SAPIENZA
Università di Roma
Facoltà di Scienze Matematiche Fisiche e Naturali

DOTTORATO DI RICERCA
IN GENETICA E BIOLOGIA MOLECOLARE

XXIX Ciclo
(A.A. 2015/2016)

# *Mechanisms by which nuclear A-type lamins are downregulated in neuroblastoma.*

Dottorando
Ilaria Iannetti

Docente guida
Dr Igea D'Agnano

Tutore
Dr Emanuele Cacci

Coordinatore
Prof. Silvia Bonaccorsi

# INDEX

# GLOSSARY

**Cancer:** any disease in which abnormal cells divide without control with the potential to invade nearby tissues or spread to other parts of the body through the blood and lymph systems.

**Chromatin:** complex of macromolecules consisting of DNA, RNA and proteins that forms chromosomes within the nucleus of eukaryotic cells.

**Epigenetics:** the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence. External modifications to DNA that turn genes "on" or "off".

**Gene expression:** the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA.

**Histones:** alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes.

**Laminopathies:** a group of rare genetic disorders caused by mutations in genes encoding proteins of the nuclear lamina.

**Lamins:** fibrous proteins providing structural function and transcriptional regulation in the cell nucleus. Nuclear lamins interact with membrane-associated proteins to form the nuclear lamina on the interior of the nuclear envelope.

**Methylation:** the chemical reaction consisting of the addition of a methyl group on a substrate.

**microRNA:** small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that functions as post-transcriptional regulators of gene expression.

**Neuroblastoma:** a type of cancer that starts in certain very early forms of neural cells found in an embryo or fetus. This type of cancer occurs most often in infants and young children.

**Nervous System:** the part of an animal's body that coordinates its actions and transmits signals to and from different parts of its body.

**Nuclear Envelope:** a highly regulated membrane barrier that separates the nucleus from the cytoplasm in eukaryotic cells.

**Nuclear Lamina:** dense fibrillar network inside the nucleus and lined with the Inner Nuclear Membrane of most eukaryotic cells.

**Polymerase chain reaction:** technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

**Transcription Factors:** proteins involved in the process of converting, or transcribing, DNA into RNA.

# SUMMARY

Lamin A/C are essential components of the nuclear lamina. Together with B-type lamins and other structural components, they form the network which is located in the inner side of the nuclear membrane. It is now clear that lamins exert many different functions within the cell, most of them still largely unknown. Lamin A/C are absent in embryonic stem cells but are expressed in the majority of adult cell types, thereby suggesting their crucial role in differentiation. In addition, their expression is reduced or absent in several human malignancies, even though their role in the tumorigenesis has not been completely characterized yet. A down regulation of Lamin A/C could result in increased nuclear deformability thereby facilitating transit of cells through the capillaries and favouring cell invasive capacity. In a previous paper we demonstrated that LMNA gene knock-down inhibits differentiation in a cellular model of neuroblastoma and increases tumor progression. In this project I investigated some of the possible mechanisms by which Lamin A/C is down-regulated in neuroblastoma cells. As cellular models I employed two neuroblastoma cell lines showing different level of Lamin A/C expression (LAN-5 and SH-SY5Y cells). The analysis of the nascent transcripts of LMNA gene in both cell lines demonstrated that the transcriptional rate of this gene was reduced in the LAN-5

cells, indicating that the protein is regulated at transcriptional level. In addition, I observed that there is a minor recruitment of Sp1 transcription factor on the LMNA promoter of LAN-5 cells, as evaluated by ChIP assay. Moreover, even though the chromatin configuration upstream the LMNA gene promoter is open in both cell lines, as evidenced by a similar enrichment of the H3K4me3 histone marker in both cell lines, we found a different configuration in the coding sequence region (CDS), as shown by a reduced enrichment in LAN-5 cells of the histone marker H3K36me3 which is associated with transcriptional activation. We then tried to exogenously express the LMNA gene in LAN-5 cells, in which the protein is not detectable. In spite of a very high upregulation of LMNA gene, I did not observed any detectable expression of Lamin A/C protein. I hypothesized that the protein could be regulated by a miRNA-mediated post-transcriptional mechanism. An in silico analysis of the miRNAs predicted by miRWalk database to significantly target LMNA transcripts, have evidenced a unique miRNA, the has-miR-539-5p. However, the inhibition of the expression of such miRNA was not able to rescue the expression of Lamin A/C protein in LAN-5 cells. At the moment I can conclude that the regulation of Lamin A/C protein in neuroblastoma cells is mainly to be ascribed to a transcriptional mechanism. However, I cannot exclude the existence of an

additional regulation mechanism occurring at post-transcriptional and/or post-translation level that have to be further investigated.

# 1 INTRODUCTION

## 1.1 Nuclear lamins

In eukaryotes, the inner surface of the NE is lined with a network of filamentous proteins forming the nuclear lamina (NL). The NL consists of two components, Lamins and nuclear lamin-associated membrane proteins. Lamins are type V intermediate filaments (IF). They are divided in "A-type" lamins, lamin A and C, expressed during development only in differentiating cells, and "B-type" lamins, lamin B1 and B2, expressed also in stem cells and essential for cell viability (*Osmanagic-Myers et al., 2015*). The main function of the NL is to provide mechanical support to the NE by forming a scaffold-like meshwork underlying the inner nuclear membrane. The nuclear lamin-associated mediate the attachment of the nuclear lamina to the nuclear envelope. The interactions with these proteins are pivotal for mitotic nuclear envelope disassociation and reassembly, nucleus size and shape, as well as correct positioning and spacing of the Nuclear Pore Complexes (NPCs). A-type lamins also interact with DNA directly though the C-terminus tail (*Stierle et al., 2003*) or through lamina-associated proteins, such as LAP1 and LAP2b or nucleoplasmic LAP2a, and this association guides nuclear peripheral positioning and keeps constitutive silencing of heterochromatin (*Foisner and Gerace, 1993*). Recently, the interaction between LAP2a and lamin A was

demonstrated to be capable of promoting proliferation of human fibroblasts. Among these Inner Nuclear Membrane associated or nucleoplasmic interacting partners, a special group containing LEM domains (LAP2, emerin, MAN1 domain) can directly interact with BAF (Barrier-to-autointegration factor), suggesting a potential role of A-type lamins in the regulation of chromatin modification and/or remodeling (*Gruenbaum and Foisner 2015*). These lamins also interact with transcription factors, including Rb, SREBP1, MOK2 (*Dreuillet et al., 2002*) and c-Fos, to regulate their activities (*Ivorra et al., 2006*).

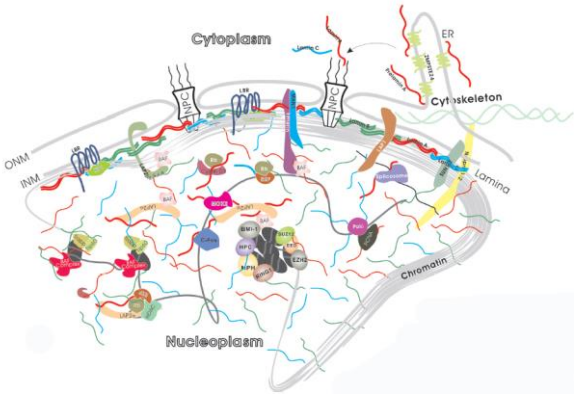



**Fig. 1. Nuclear envelope architecture.** Nuclear envelope proteins that are bound to the lamina include nesprin, emerin, lamina-associated proteins 1 and 2 (LAP1 and LAP2), the lamin B receptor (LBR) and MAN1. Barrier to autointegration factor (BAF) is a chromatin-associated protein that also binds to the nuclear lamina and several of the aforementioned nuclear envelope proteins. Heterochromatin protein 1 (HP1) binds both chromatin and the LBR. ONM, outer nuclear membrane (Liu et al, 2007).

## 1.2 A-type lamin gene

Number and complexity of A-type lamins increase with metazoan evolution. For example, *Caernorhabitis elegans* has unique gene for lamins, *lmn-1*, expressed in all cells except in mature sperm (*Liu et al., 2000*); *Drosophila melanogaster* has two genes codifing for lamins: *lmn Dm0* and *lmn C*, homologous to A type and B type of vertebrate lamins respectively (*Bossie and Sanders, 1993*). LMNA is the gene codifying for A-type lamins, it is on chromosome 1 in 1q22 locus, and the gene is formed by 24Kb and 12 exons. The exon 1 coding for the N-terminal domain and the initial portion of the rod domain, the exons 2, 3, 4, 5 and 6 for the whole rod domain, the exons 7, 8 and 9 for the C-terminus domain including the NLS sequence (*Dutta et al., 2016*). And the exon 10 have an alternative splice-site that create the 4 different isoforms of A-type lamins existent: Lamin A, Lamin AΔ10, Lamin C and C2 (*Furukawa et al., 1993*). While the isoforms A, AΔ10, C are regulated during development and are mainly expressed in differentiated cells (*Gruenbaum and Foisner 2015*) the C2 isoforms are expressed only in the germ line (*Alsheimer et al., 1999*). Mammals have another 2 two genes for lamins, codifying for B-type lamins: LMNB1 and LMNB2. LMNB1 is on chromosomes 5 in locus 5q23.2-q31.3 and codifying for lamin B1, LMNB2 genes is on 19p13.3 locus and codifying for lamin B2 and

lamin B3 (*Lin and Worman, 1995; Dechat et al., 2008*). Lamin B1 and lamin B2 are regulated and expressed in both embryonic and somatic cells during development whereas lamins B3 is regulated and expressed only in spermatocytes (*Gerace and Blobel, 1980; Hutchison, 2002*). The increasing complexity of lamins seems to be correlated with the increase in the efficiency of nuclear envelope disassembly during mitosis (*Cohen et al., 2001*). Mutations in genes for these proteins are associated with several inherited diseases.

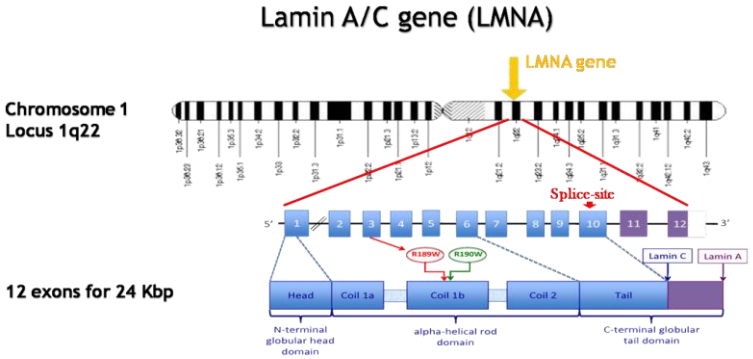



**Fig. 2. A-type lamin gene location and structures** (*Botto et al., 2010*).

## 1.3 Lamin A/C protein structures

Lamin A/C proteins have a molecular weight of 60-75 kDa and a length of 30-100 nm. These proteins are characterized by a not structured N-terminus domain, an internal α-helix domain, called rod domain (*Fisher et al., 1986*), and at the C-terminus there are

three structures that distinguish lamins from the other members of intermediate filaments: a nuclear localization sequence NLS, not present in the other members of IF, an immunoglobulin fold, and a conserved CAAX box, which consists of a cysteine, two aliphatic amino acids and any COOH-terminal amino acid (*Davies et al., 2009; Hermann and Strelkrov, 2011*). The α-helix domain is necessary to form the coiled-coil dimers, four coiled-coil dimers, separated by flexible linkers regions, are associated longitudinally head-to-tail to form the highly structured reticular polymer called Nuclear Lamina (*Hermann and Aebi, 2004)*. The C-terminus domain is an important structure, which allows to extensive post-translational modification of these proteins to become mature lamins. The modifications consist in farnesylation, carboxymethylation, sumoylation, ADP-ribosylation (*Vidak and Foisner, 2016*). Phosphorylation of lamins at specific sites is instead required at the onset of mitosis to drive disassembly of the nuclear lamina (*Burke and Gerace, 1986; Foisner and Gerace, 1993; Mall et al., 2012*).


**Fig. 3. Schematic structure of Lamin A/C proteins.** Prelamin A is composed of a 28-residue positively charged N-terminal globular domain, a central a-helical rod-like, a conserved nuclear localization signal (NLS), one Ig-like

structure formed by approximately 116 residues on the C-terminus and a positively charged C-terminal globular domain.

## 1.4 Regulation of LMNA gene expression

### *1.4.1 Transcriptional regulation*

The coordinated expression of genes drives the cellular processes. This coordination is in part regulated by interactions between proteins, called transcription factors (TFs) and sequence-specific DNA elements, called TF-binding sites (TFBS). These interactions regulate gene transcription and expression. The expression of LMNA gene is mostly regulated by transcriptional mechanisms, in fact the mRNA of LMNA is detected in differentiated cells while is absent in embryonic cells (*Hamid et al., 1996*). The LMNA gene of rat has a proximal promoter, which contain several important gene activation regions, a GC region at -101, a binding site for Sp1/Sp3 transcription factors (TFs), and another region at -7, which is a binding site for the transcription factor AP-1. Finally, TATA-box is at -33*(Tiwari et al., 1998)*. The importance of these regions has been demonstrated by the fact that their mutation significantly reduces the activity of the promoter. The Sps transcription factors known are Sp1, Sp3 and Sp4. They preferentially bind regions rich in GC and GT, with greater affinity for the GC regions (*Suske, 1999*). While Sp1 and Sp3 are present

in all tissues, Sp4 is mostly present in nervous tissue. The Sps transcription factors can bind the promoter and operate in an independently manner, otherwise cooperate synergistically (*Bigger et al., 1997*). It was also shown that the GC regions can be controlled by proteins regulated by signaling pathway of retinoic acid (RA) (*Okumura et al., 2000*). It was recently identified a new GT region at -55 required for optimal activation of the promoter. This region is bound by Sps, which recruit the coactivator CBP/p300, showing acetyl transferase activity of H3 and H4 (*Janaki Ramaiah and Parnaik, 2006*). The promoter of the rat LMNA gene is conserved in humans, with a high homology of GC regions, TATA-box and AP-1 (*Nakajima and Abe, 1995*) and since these elements flank the TATA-box region, it is likely that they help the binding of the transcription factors, necessary for the assembly of transcription complexes (*Muralikrishna and Parnaik., 2001*).

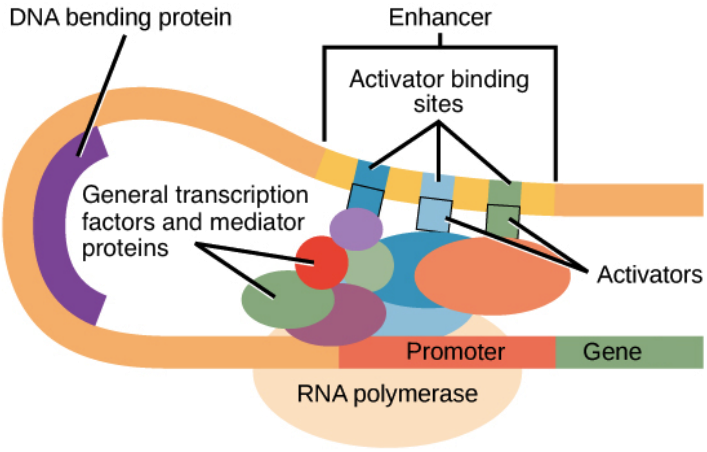



**Fig.4. Schematic representation of transcription activation by transcription factor complexes.** OpenStax College, Biology (CC BY 4.0).

## *1.4.2 DNA methylation*

Research over the past has focused on different molecular mechanisms that mediate epigenetic phenomena. The term "epigenetic" is used to describe stable and heritable modifications that do not involve mutations of DNA sequence, but generate functional changes in terms of accessibility of DNA to basal replication and transcriptional machineries. One of most important epigenetic phenomena is the DNA methylation of CpG regions. In this mechanism, a methyl group is attached to the 5th atom in the 6-atom ring creating 5-methylcytosine by particular enzymes called DNA methyltransferases (DMNTs). The presence of methyl groups on cytosine sterically interferes with the binding of

transcription factors to consensus sequences, therefore the transcription complex in not recruited, while are being recruited gene silencing proteins for the chromatin packaging. Agrelo et al. demonstrated that the hypermethylation of the LMNA promoter is correlated with gene silencing of the region, because the presence of the mRNA and the protein is no longer observed. They observed the phenomenon in lymphoma and leukemia models. Given that A-type lamins have an important role either in chromatin protection from damages or in regulation of gene expression, it is very likely that the absence of nuclear lamins is related to a lack of cell differentiation and cancer process progression (*Agrelo et al., 2005*).

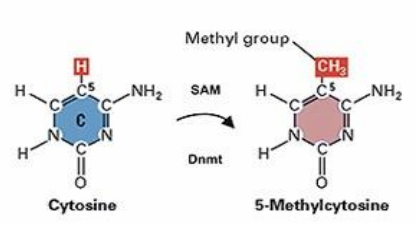



**Fig.5. Skeletal structure for the methylation of methylcytosine to form 5-methylcytosine, using S-adenosylmethionine as the source of the methyl group and giving S-adenosyl-L-homocysteine as product.** (Human Genetics Unit, Medical Research Council of UK).

## *1.4.3 Histone modifications*

Other epigenetic mechanisms, which can affect the rate of gene expression, are the covalent post translational histone

modifications. Histones proteins can undergo a variety of post translational modification (PTMs) some of which are methylation, acetylation, ubiquitylation, sumoylation and phosphorylation on specific amino acid residues. These histone modifications occur at several degrees, for example, methylation can be of monomethyl (me), dimethyl (me2) and trimethyl (me3). Histone PTMs are added on and removed from histones by enzymes, called "writers" and "erasers" respectively. Histone acetyltransferases (HATs), histone methyltransferases (HMTs) and histone kinases are the examples of "writers" which add acetyl, methyl and phosphoryl groups, whereas histone deacetylases (HDACs), histone demethylases (HDMs) and histone phosphatases are examples of "erasers" which remove acetyl, methyl and phosphoryl groups, respectively. The mechanism behind the regulation of key cellular processes by histone post-translational modifications is not fully understood; however, it can be generalized into two categories. First, the addition of any PTM on histone proteins affects inter/intra-nucleosomal interactions and their binding to DNA by steric hindrance or charge interactions. Second, addition of these PTMs to histone proteins inhibits or facilitates the binding of various proteins to chromatin. These mechanisms allow a vast range of flexibility in regulating chromatin dynamics and signaling transmission and thereby regulating the gene expression.

As an example of first mechanism, histone acetylation is proposed to be associated with chromatin remodelling and transcriptional activation, H4K16ac inhibits the formation of compact 30 nm fibers and higher order chromatin structures. As an example of second mechanism, evolutionarily conserved specialized proteins, termed "histone readers," possess the ability to specifically bind certain histone modifications and affects a defined nuclear process such as transcription, DNA repair and replication, *etc.* For example, through its evolutionary conserved chromodomain heterochromatin protein 1 recognize and gets recruited to H3K9me3 and H3K27me3 and leads to the formation of compact chromatin which in turn inhibits the access of the transcriptional machinery. Such complicated and multilayered regulatory mechanisms of cellular processes through histone modifications have led to the hypothesis of "histone code" where a set of histone variants and modifications together perform a specific function. However, due to its complexity histone code is still not fully understood. Further, the status of one histone modification also regulates that of another by cross-talk and affects chromatin remodeling and gene expression. Cross-talk between H3S10ph and H3K14ac, H2Bub and H3K4me and H3K4ac and H3K4me3 and H3K14ac are few prominent examples regulating gene expression (*Khan SA et al., 2015*).

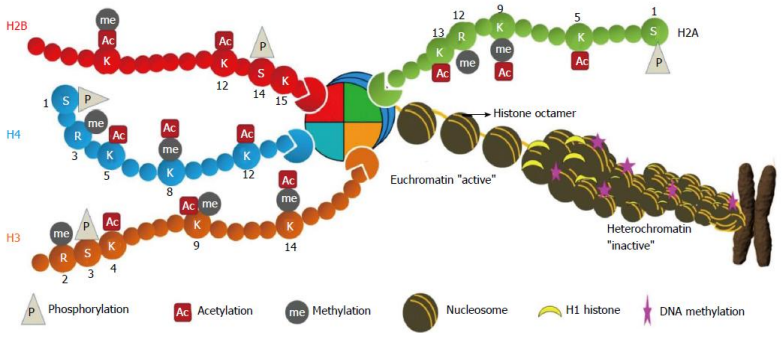



**Fig. 6. Histones modifications and chromatin architecture**. Histones within the nucleosome (two each of H2A, H2B, H3 and H4) undergo numerous post-translational modifications at their N-terminal tails, which protrudes from the nucleosome. Further folding of nucleosome with linker histone H1 creates a spiral structure, the heterochromatin leading to metaphase chromosome. These modifications directly regulate the chromatin structure and thus DNA-mediated cellular processes. The diagram indicates some modifications at specific residues: M: Methylation; A: Acetylation; P: Phosphorylation. (*Khan et al., 2015*).

## *1.4.4 Post-transcriptional regulation*

The mRNA of LMNA is initially unique, and it is constituted by 12 exons codifying for the several domain of the protein, there are two polyadenylation signals located on exon 10 and exon 12, furthermore the exon 10 have an alternative splice site. During mRNA maturation alternative splicing mechanism create the four existent different isoforms of lamin A/C: lamin A, lamin AΔ10, lamin C and lamin C2 (*Liu et al., 2008*). All isoforms share the

nucleotide sequence from exon 1 to exon 9. The isoform A contains 9 exons, 90 bases at the 5'exon 10, and exons 11 and 12; the C isoform contains 111 bases of exon 10 but not exons 11 and 12; isoform AΔ10 does not contain exon 10; and the isoform C2 has an alternative N-terminal encoded from the middle region of intron 1 (*Barbie et al., 1996*).

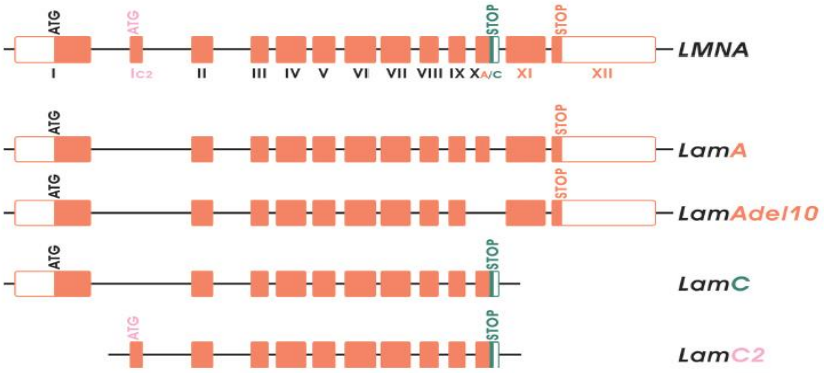



**Fig. 7. *LMNA* and A-type lamins.** The *LMNA* gene has 12 exons and contains two polyadenylation signals, which are located in exon 10 and 12, and are utilized by lamin C/C2 and lamin A/AΔ10 respectively. As a consequence of alternative splicing, the first exon in lamin C2 is different from other A-type lamins, and lamin AΔ10 shares identical exons with lamin A but lacks exon 10. (*Liu et al., 2008*).

## *1.4.5 Regulation by microRNAs*

Another mechanism of LMNA gene regulation could be the regulation by microRNAs (miRNAs). miRNAs are evolutionally

conserved, small non-coding RNAs that are about 19-22 nucleotides (nt) long. miRNAs modulate gene expression by repressing their targets' translation or inducing mRNA degradation through binding to the complementary sequence in target messenger RNA. The biogenesis of miRNAs is a complex multi-step process that starts in the nucleus and ends in the cytoplasm of cells. Most miRNAs are transcribed as long monocistronic or polycistronic primary transcription units (pri-miRNA) by RNA polymerase II. Typically, a pri-miRNA is characterized by a hairpin structure, containing a double-stranded (ds) RNA stem of ~33 base pairs (bp), a terminal loop, and single-stranded (ss) RNA flanking regions. The stem-loop structure contains the miRNA in the 5' or 3' half of the stem. The pri-miRNA is cleaved in the nucleus by a protein complex (the "microprocessor complex") consisting of several proteins including the RNase III enzyme, Drosha and its co-factor DGCR8. DGCR8 functions as a molecular anchor and defines the binding site for the microprocessor, while Drosha cleaves the RNA approximately 11 bp from the ss-dsRNA junction, producing the shorter, ~ 65-70-nucleotide long hairpin pre-miRNA. Following completion of this nuclear processing step, the pre-miRNA is exported from the nucleus to the cytoplasm by the Exportin-5. Here, the pre-miRNA is cleaved by another RNAse III enzyme called Dicer. Dicer cleaves ~22 nt from the pre-existing end of the pre-miRNA, producing ~22 nt double-stranded

RNA molecules. One of the two strands (the guide strand or mature miRNA) is, upon selection by thermodynamic properties, loaded on an Argonaute (Ago) protein, the main constituent of the RNA-Induced Silencing Complex (RISC). The other strand (passenger strand) is degraded. The mature miRNA sequence guides the RISC complex to recognize and target partial complementary mRNA sequences, primarily within the 3'-untranslated region (3'UTR) (*Kim et al., 2009; Siomi et al., 2009; Guarnieri et al., 2008*).

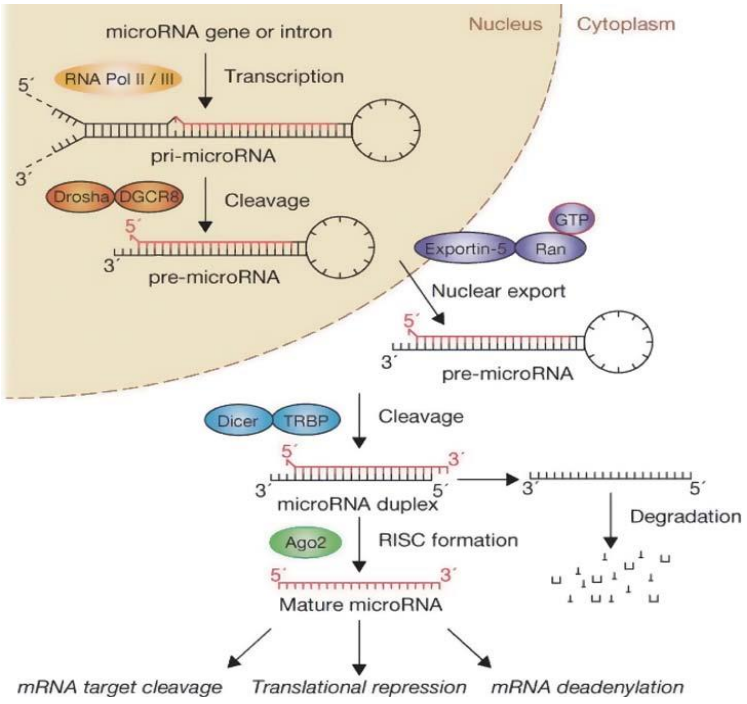


**Fig. 8. miRNA biogenesis pathways and function in gene regulation.** (*Winter et al., 2009*)**.**

The mechanisms of miRNA-mediated post-transcriptional regulation on target mRNAs include blocking the translation and/or inducing the degradation of mRNAs by deadenylation and decapping processes. No matter which mechanism predominates, the overall output is the reduced amount of protein encoded by the target messenger (*Bartel, 2009*).

As miRNAs tend to target many different mRNAs, and each mRNA may contain several to hundreds of different miRNA binding sites, it is obvious that the miRNA-mRNA regulatory network is extremely complex. It has been estimated that 30-60 % of all human genes are regulated by miRNAs (*Lewis et al., 2005; Friedman et al., 2009*). miRNA regulation is involved in almost all physiological processes, including stem cell self-renewal, differentiation, proliferation, metabolism, survival and death pathways (*Guarnieri et al., 2008; Huang et al., 2011*). As a consequence of this broad function, miRNA biogenesis has to be tightly controlled. Deregulated miRNA expression has been associated with a variety of diseases, including cancer. Changes in the composition of the nuclear membrane occur during the progression of several types of cancers that could be associated to miRNA deregulation (*Park et al., 2009; Weber et al., 2010*).

miRNAs are annotated and catalogued in the public-accessible web-based database miRBase (www.mirbase.org), which was founded at the Sanger Institute in England and is now managed by the University of Manchester. So far, more than 5000 mature miRNAs have been reported in humans (*miRbase, Kozomara et al., 2014*). The miRNA nomenclature is managed by miRBase and has been slightly changed with upcoming releases of the database. In general, miRNA names start with a 3-4-letter prefix to designate the species (e.g. hsa- for *Homo sapiens* miRNAs). They are further assigned by a three-letter prefix, such as miR- or let-, followed by a sequential number (e.g., miR-1). In some cases, two mature miRNAs are processed from the same stem-loop precursor, one from each arm, and are accordingly designated by an additional suffix "-5p" (for that released from the 5'-arm) and "-3p" (for that released from the 3'-arm); e.g., miR-199a-5p and miR-199a-3p. The star-forms (miR*), previously used for minor forms, have been "retired" according to the latest nomenclature convention (*Kozomara et al., 2011*).

### *1.4.6 Post-translational regulation*

Lamins before being incorporated in the nuclear lamina undergo post translational maturation processes at the carboxyl domain, including covalent modifications such as phosphorylation,

farnesylation and sumoylation (*Prokocimer et al., 2009*). The precursor peptide of lamin A/C, called prelamin, is processed by several cleavage that will culminate in the assembly of the reticular polymer of NL (*Moir and Goldman, 1993*), the half-life of the molecular precursor is 90-100 minutes and in about 4 hours is acquired and incorporated into the nuclear network (*Gerace et al., 1984*). The precursor protein has a farnesylation signal (*Clarke, 1992*) on the CAAX-box (*Fisher et al., 1993*), this feature is absent in lamin C (*Horton et al., 1992*). Then, ZMPSTE24, also known as farnesylated-proteins converting enzyme 1 (FACE-1), cleaves behind farnesylcysteine to release –AAX (*Corrigan, 2005*). This is followed by methylation on the same cysteine by isoprenylcysteine methyltransferase (ICMT). Finally, ZMPSTE24 or other unidentified enzymes catalyze a second proteolytic cleavage to remove an additional 15 amino acids on the C-terminus (*Vidak and Foisner, 2015*). The mature lamin A is approximately 2 kDa less than prelamin A (*Agarwal et al., 2003; Corrigan et al., 2005*). The role of this last step is not clear, however the reaction of first isoprenylation is required for all next maturation processes (*Holz et al., 1989*). It is not yet clear where the precursors of lamins are processed within the cell, some authors have concluded that the maturation occurs at the level of cytoplasm (*Kitten and Nigg, 1991*), others affirm that lamins are processed after assembly in the network (*Gerace et al., 1984*). It has been shown that lamins are

present within the nucleus in particular in intranuclear *foci*, as well as in perinuclear regions (*Sasseville and Raymond, 1995*).

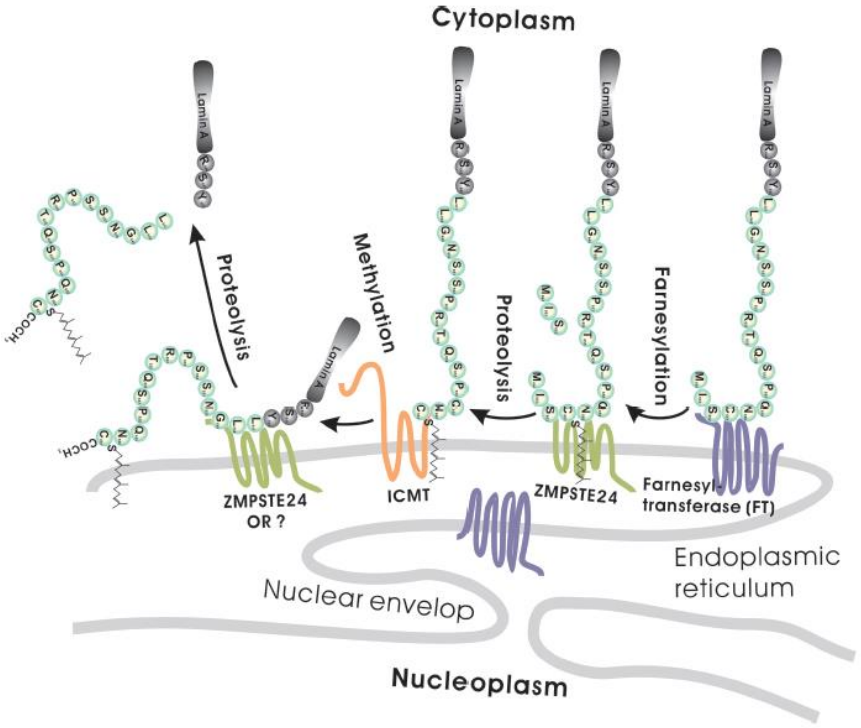



**Fig.9. Post-translational processing of prelamin A.**Lamin A is firstly synthesized as a precursor with conservedCAAX motif on the C-terminus. Its maturation requires mainly four steps. Firstly, the cysteine residue of CAAX is farnesylated by farnesyl transferase (FT) located on the endoplasmic reticulum (ER). Secondly, Zmpste24 mediates a proteolytic cleavage after farnesyl-cysteine to release the last three amino acids (AAX). Then the exposed farnesylcysteine is further methylated by ICMT. Finally, Zmpste24 mediates the second proteolytic cleavage to remove an additional 15 amino acids (*Liu et al., 2008*).

## 1.5 Lamin functions

The main function of the nuclear lamina is to provide support to the NE, by forming a scaffold-like meshwork underlying the inner nuclear membrane determining the overall shape of the nucleus (*Gruenbaum and Foisner, 2015*). Recent reports showed that lamins define the mechanochemical properties of the nucleus (*Osmanagic-Myers et al., 2015*); lamin A is responsible for nuclear stiffness, and B-type lamins for nuclear elasticity (*Buxboim et al., 2014; Swift et al., 2013*). Besides their mechanochemical role, lamins have a multitude of additional functions, including chromatin organization, gene regulation, DNA repair, and (mechano-) signaling (*Amendola and van Steensel, 2014; Andres and Gonzalez, 2009; Dechat et al., 2010; Dittmer and Misteli, 2011; Gruenbaum and Foisner, 2015; Ho and Lammerding, 2012*). The nuclear lamina is physically connected to the cytoskeleton via the linker of nucleoskeleton and cytoskeleton (LINC) complex, which plays a crucial role in mechanotransduction (*Crisp et al., 2006*). The LINC complex is comprised of SUN (Sad1 & UNC-84) domain proteins spanning the Inner Nuclear Membrane (*Ketema et al., 2013*) and is connected to nesprins (also referred to as Syne, Myne, and NUANCE) at the Outer Nuclear Membrane (*Chang et al., 2015*). Different isoforms of nesprin also bind actin and microtubules and cytoplasmic intermediate filaments (*Mèjat et al.,*

*2010*). Silencing or depleting the components of the LINC complex results in nuclear envelop deformities, thereby suggesting that these components are necessary to maintain proper shape and structure of the nucleus in response to mechanical forces (*Cain et al., 2014*). The many roles of the lamins are mediated by their interactions with several proteins of nuclear periphery and nucleoplasm such as histone dimers H2A/H2B, the Retinoblastoma protein (Rb is a tumor suppressor and a negative cell cycle regulator), LAP-2α, Erk kinase-1/2, nuclear actin, PCNA and nuclear pore proteins *(Hutchison, 2002*). Lamin A/C also plays essential roles in RNA splicing. Using a nucleoplasmiclamin A/C specific monoclonal antibody, LA-2H10, which only stained speckles in interphase nuclei without labeling the nuclear rim, lamin A/C was reported to co-localize with RNA splicosome, indicating its potential role in mediating RNA metabolism (*Kumaran et al., 2002*). Several evidences indicate that lamins are also necessary for the replication of DNA, for example Lamins are necessary for a correct localization of replication factors such as PCNA, during the elongation phase of DNA replication (*Moir et al., 2000*). Lamins also have a role in epigenetic control of gene expression because regulate the nuclear chromatin status. It is known that lamins bind directly, through the carboxyl terminus, or indirectly, by binding other associated proteins, heterochromatin (*Schirmer and Foisner, 2007*). It has been proposed a nuclear

architecture model in which lamins are important factors for the positioning of chromosomes in the nucleus (*Reddy et al., 2008*). Recently studies demonstrated that there is a crosstalk between Lamin A/C protein and the Polycomb group (PcG) a epigenetic repressors that control a large number of target genes during differentiation (*Lanzuolo and Orlando, 2012*). In the nucleus, PcG proteins form microscopically visible foci (*Cmarko et al., 2003*), and high-throughput data together with microscopy analysis have revealed specific organization of their targets in chromatin loops (*Lanzuolo et al., 2007; Bantignies et al., 2011*). In human LMNA-null cells nuclear positioning of the PcG protein is alterated (*Masny et al., 2004*) demonstrated that lamin A/C can modulate transcription through the regulation of PcG protein epigenetic factors. On a global level, lamins can influence gene expression because they provide the structural scaffolding for the organization of the transcriptional complex regulated by RNA polymerase II (*Dittmer et al., 2014*). It has also been described that A-type lamins can interact with transcription factors in different ways, capturing these molecules in inactive complex, by altering the post-translational modifications, which are important for their function (*Gonzalez et al., 2011*).

## 1.6 Lamins and Laminopathies

Since 1999, the year in which it was discovered that a hereditary mutation in LMNA gene caused the Emery-Dreifuss muscular dystrophy (EDMD) (*Bonne et al., 1999*), more than 450 diseases have been associated with mutations of Lamin A/C, and are collectively referred to the term *Laminopathies*. These disorders include Hutchiston-Gilford progeria syndrome (HGPS), dilated cardiomyopathy with conduction defect (DCM-CD), the familiar partial dystrophy Dunningan (FPLD), the Charcot-Marie Tooth (CMTS) and many others (*Worman et al., 2015*). The high degree of tissue-specificity of Lamins A/C and the broad range of diseases caused by mutations in the same gene appear to be not clear (*Shreiber and Kennedy, 2013*). Many hypothesis have been postulated to explain this: the 'structural hypothesis' suggests that lamin mutations increase nuclear fragility resulting in cell death (*Simon and Wilson, 2013*). The 'gene regulation hypothesis' proposes that lamin mutations interfere with tissue-specific genes (*Peric-Hüpkes et al., 2010*). A third hypothesis proposes that lamin mutations impair stem cell function such as differentiation bringing to defects in proliferation and survival (*Chen et al., 2013*). These hypotheses are not mutually exclusive, and it is likely that laminopathies arise from a combination of defects in lamin functions.

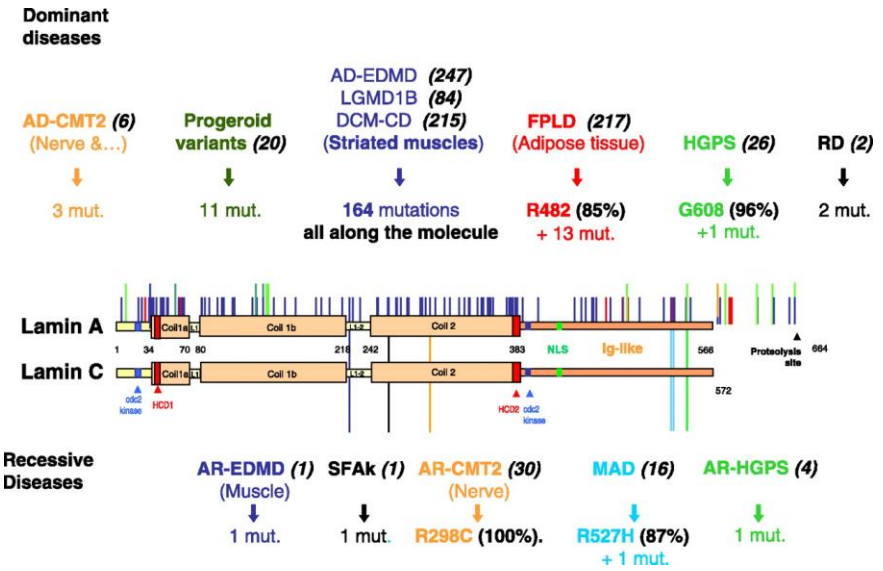



**Fig.10. Mutations of LMNA gene and laminopathies.** (*Broers et al., 2006*).

## 1.7 Lamins and Cancer

Recently it was reported that lamins are implicated in many types of human cancer, it is clear that A-type lamins are involved in the progression of ovarian, lung, prostate, breast, colon and blood cancer (*De Las Heras et al., 2012*). Lamins are involved in the cell survival process in two ways: first, they interact with the linker protein nesprins, a "bridge" between the nucleoskeleton and the cytoskeleton, that transduce the mechanical stress signals coming from the plasma membrane to the nuclear membrane (*Padmakumar et al., 2004*). Secondly, they interact with many

molecules that regulate cell growth. These proteins have a particular binding domain for lamins, called LEM domain, a globular module of approximately 40 amino acids, which is mostly found in the nucleoplasmic portions of metazoan inner nuclear membrane proteins (*Wagner and Krohne, 2007*), and include emerin, the HND1 protein and LAP2α (*Dechat et al., 2000*). The lamin A/emerin complex regulates nuclear accumulation of β-catenin and the loss of this interaction involves the deregulation of β-catenin (*Markiewicz et al., 2006*), furthermore HND1 and lamin A interact with the rSMAD (*Lin et al., 2005*), and the complex lamin A/LAP2α binds the dephosphorylated form of Rb in the nucleus. Lacking of the interaction between these molecules causes a premature entry into S phase and thus the cell growth in fibroblasts (*Markiewicz et al., 2002*). In colo-rectal cancer it was observed that the altered expression of lamins A/C, and especially a decreased production of the protein, is associated with increased aggressiveness of the cancerous cells and the risk of relapse (*Belt et al., 2011*). The higher invasiveness of these cells can be attributed to the fact that lamin A/C has a rule in the metabolic pathway of plastins, a actin binding proteins actin (*Delanote et al., 2005*). Misregulation of this pathway could mediate down regulation of E-cadherin and cell adhesion proteins, phenomenon that is closely related to the formation of metastases and tumor aggressiveness (*Foran et al., 2006*). Another possible mechanism

that the down regulation of Lamin A/C could result in increased nuclear deformability thereby facilitating transit of cells through narrow constrictions such as capillaries, and therefore invade surrounding tissue to form metastases (*Sakthivel et al., 2016*). In a recent work conducted in neuroblastoma cell lines it has been shown that down-regulation of Lamin A/C in human neuroblastoma cells leads to a more aggressive tumor phenotype, with increased invasive ability and drug-resistance to anticancer treatments (*Maresca et al., 2012*).

## 1.8 Neuroblastoma

Neuroblastoma (NB) is an extra-cranial heterogeneous tumor of the sympathetic nervous system (*Esiashvili et al., 2009*) and arises from developing sympathetic nervous system. Most primary tumors localize in the abdomen, the adrenal gland, or lumbal sympathetic ganglia (*Bottino et al., 2014*) The first description of pediatric tumors under the term "neuroblastoma" was made by Dr. James Homer Wright of the Massachusetts General Hospital as early as 1910 (*Modak et al., 2010*). More than 50% of these tumors occur in children less than 2 years of age. The incidence of neuroblastoma has increased in recent years and it continues to carry a poor prognosis in children over two years of age with a

survival of only 38% (*Cotterill et al., 2001*). Today approximately 120 new cases are diagnosed in Italy each year, rendering this tumor the most common extra-cranial pediatric cancer (www.neuroblastoma.org). In 95% of cases, NB is diagnosed before the age of 5 years where it leads to 15% of all cancer-related fatalities in infancy and childhood (*Mueller et al., 2009*). Despite a century of extensive clinical and basic research efforts, the biology and clinical progression of the disease continues to present a multi-faceted clinical enigma in pediatric oncology. On the one hand, neuroblastoma accounts for disproportionate morbidity and mortality among the cancers of childhood; on the other hand, it is associated with one of the highest proportions of spontaneous and complete regression of all human cancers(*Brodeur, 2003*). The most common site for primary NB tumors is the adrenal medulla; however, tumors can arise anywhere along the sympathetic branch of the autonomic nervous system (*Alam et al., 2009*). At presentation, the disease can be limited to a single organ, locally or regionally invasive, or widely disseminated; more than 50% of cases are metastatic at presentation (*Alam et al., 2009*). The most common metastatic sites are lymph nodes, bone marrow, bone, and liver (*Alam et al., 2009*).

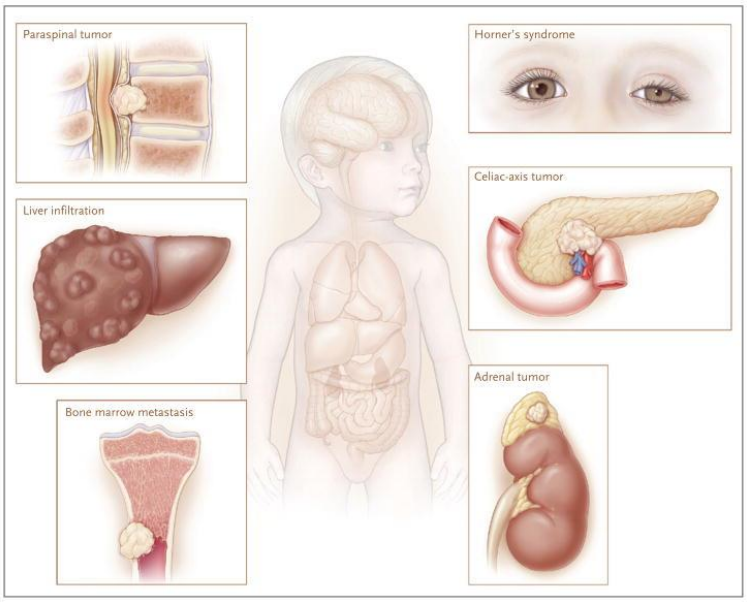



**Fig. 11. Clinical Presentations of Neuroblastoma.** Neuroblastoma is a childhood cancer that is diagnosed at a median age of about 17 months. Tumors can arise anywhere along the sympathetic nervous system, with the majority occurring in the adrenal medulla. Primary tumors in the neck or upper chest can cause Horner's syndrome (ptosis, miosis, and anhidrosis). Tumors along the spinal column can expand through the intraforaminal spaces and cause cord compression, with resulting paralysis. Although many lower-stage neuroblastomas are encapsulated and can be surgically excised with little chance of complications, higher-stage tumors often infiltrate local organ structures, surround critical nerves and vessels such as the celiac axis, and are largely unresectable at the time of diagnosis. Neuroblastomas typically metastasize to regional lymph nodes and to the bone marrow by means of the hematopoietic system. Tumor cells metastatic to marrow can infiltrate cortical bone. Neuroblastomas also can metastasize to the liver, most notably in patients with stage 4S tumors, in whom involvement can be extensive; however, transient and

complete regression often occurs with no intervention other than supportive care. (*Maris et al., 2010*).

## 1.9 Nervous system development

The human nervous system consists of the Central Nervous System (CNS), comprising the brain and the spinal cord, and the Peripheral Nervous System (PNS), which links the CNS with the body's sense receptors, muscles, and glands. The PNS is divided in two components: the somatic or skeletal nervous system, which controls voluntary movement, and the autonomic nervous system, which regulates inner organ function via the sympathetic, parasympathetic or enteric ganglia.

The nervous system originates from the neural plate, an embryonic structure evolving from the ectodermal germ layer during the third week of gestation. During the process of neurulation, the neural plate invaginates ventrally and closes in order to form the neural tube, which will give rise to the CNS. During this closure, neural crest cells originate at the interface between the closing neural tube and the dorsal ectoderm *(LaBonne and Bronner-Fraser, 1999*).

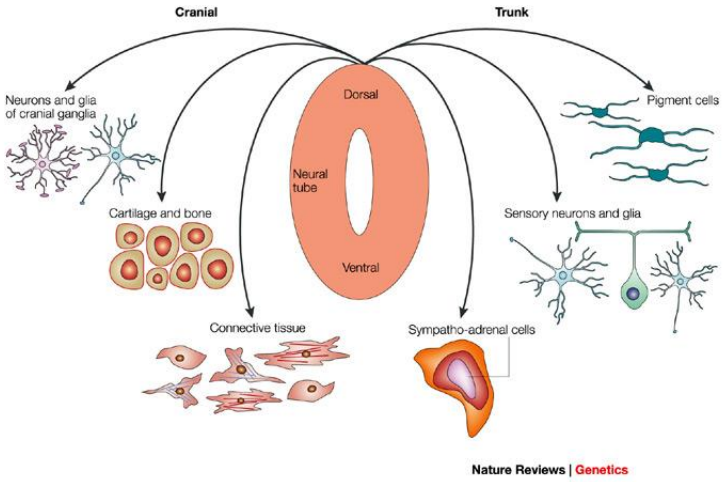



**Fig 12. Nervous system development.** After the invagination of the dorsal ectoderm, closure of the neural tube, neural crest formation at the interface of the closing neural folds and dorsal ectoderm, pluripotent crest cells migrate to their destination. (*Knecht and Bronner-Fraser, 2002*).

The neural tube is initially composed of a single layer of cells. However, as development proceeds and extensive cell division occurs, the neural tube becomes multilayered, with precursor cells dividing in the medial portion of the neural tube adjacent to the central cavity, which will give rise to ventricles. The portion of the neural tube adjacent to the ventricles becomes the Ventricular Zone (VZ) and contains neural stem cells and dividing progenitors (Greene and Copp, 2009). These stem cell populations are capable of self-renewing through symmetric cell divisions or can differentiate through asymmetric cell divisions. The neural tube

expands in the head of the embryo to form the brain and in the trunk to form the spinal cord. The early mammalian neuraltube is a straight structure. However, even before the posterior portion of the tube has formed, the most anterior portion of the tube is undergoing drastic changes. In this region, the neuraltube balloons into three primary vesicles: forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). By the time the posterior end of the neuraltube closes, secondary bulges—the optic vesicles—have extended laterally from each side of the developing forebrain. The prosencephalon becomes subdivided into the anterior telencephalon and the more caudal diencephalon. The telencephalon will eventually form the cerebral hemispheres, and the diencephalon will form the thalamic and hypothalamic brain regions that receive neural input from the retina. Indeed, the retina itself is a derivative of the diencephalon. The mesencephalon does not become subdivided, and its lumen eventually becomes the cerebral aqueduct. The rhombencephalon becomes subdivided into a posterior myelencephalon and a more anterior metencephalon. The myelencephalon eventually becomes the medulla oblongata, whose neurons generate the nerves that regulate respiratory, gastrointestinal, and cardiovascular movements. The metencephalon gives rise to the cerebellum, the part of the brain responsible for coordinating movements, posture, and balance (*Gilbert, 2000*).

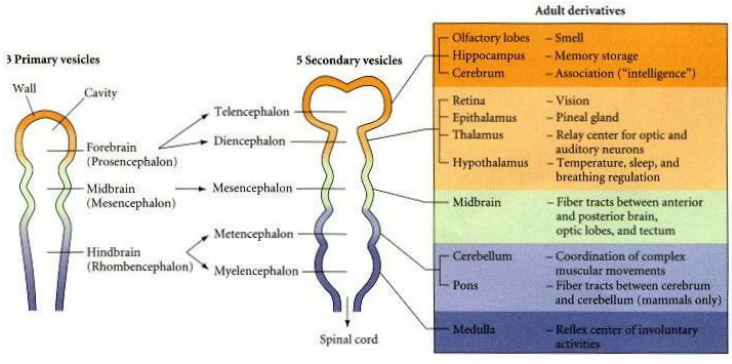



**Fig 13. Early human brain development.** The three primary brain vesicles are subdivided as development continues. On the right is a list of the adult derivatives formed by the walls and cavities of the brain. Gilbert, 2000.

The cerebellum is one of the best studied parts of the brain. The cerebellar cortex is composed of four main types of neurons: granule cells (GCs, the most numerous neurons of the entire central nervous system), Purkinje cells (PCs) and two types of inhibitory interneurons, the Golgi cells and the stellate/basket cells. The cerebellum develops over a long period, extending from the early embryonic period until the first postnatal years. The main cell types of the cerebellum arise at different times of development and at different locations. The PCs and the deep cerebellar nuclei arise from the ventricular zone of the metencephalic alar plate, whereas the GCs are added from the rostral part of the rhombic lip, known as the upper rhombic lip. The rhombic lip, is the dorsolateral part of the alar plate, and it forms a proliferative zone along the length of the hindbrain. Cells from its rostral part reach the superficial

part of the cerebellum, and form the external germinal or granular layer (EGL) at the end of the embryonic period. Granule cells are formed in the EGL. The granule cells form axons, the parallel fibres, and migrate along the processes of Bergmann glia cells (BGCs) to their deeper, definitive site, the internal granular layer (IGL). In the fetal period, the IGL is formed by further proliferation and migration of the external germinal cells. This layer, situated below the layer of Purkinje cells, is the definitive granular layer of the cerebellar cortex. A transient layer, the lamina dissecans, separates the IGL from the Purkinje cells. Ultimately, it is filled by migrating granule cells and disappears (*Rakic and Sidman, 1970*). During the inward migration of the postmitotic granule cells (16–25 weeks), the Purkinje cells enlarge and develop dendritic trees (*Milosevic and Zecevic, 1998; Miyata et al., 1999*). The EGL appears at the end of the embryonic period and persists for several months to 1–2 years after birth (*Lemire et al., 1975*).

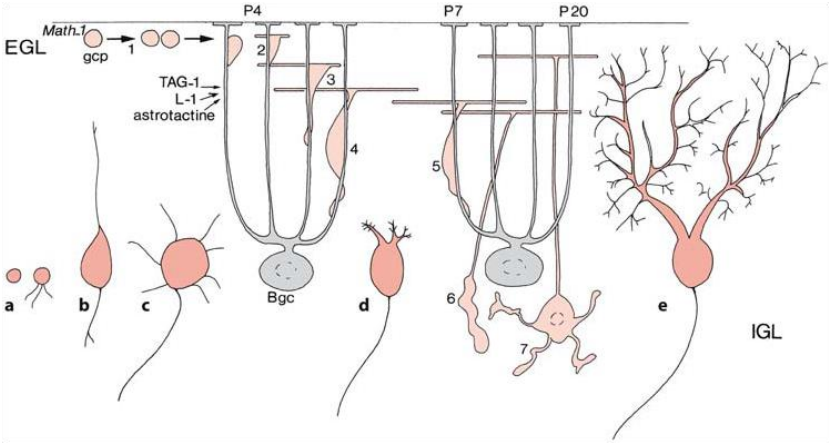



**Fig 14. Differentiation of cerebellar cortical neurons.** GCs (light red) arise (1) from granule cell precursor cells (GCPs) in the external granular layer (EGL), migrate in several steps (2–7) along the dendrites of Bergmann glia cells (BGCs) to the internal granular layer (IGL).The development of PCs (red) also involves several steps (**a**–**e**). (*Hatten et al., 1997*).

Neural crest cells delaminate from the neural tube and migrate extensively throughout the embryo to generate, differentiate, and populate numerous organs. During this migration, neural crest cells undergo differentiation and form Schwann or glial cells, melanocytes, and sympathoadrenal progenitor cells. From these sympathoadrenal progenitor cells is derived the sympathetic nervous system (SNS). SNS's special feature relates to the organism's "fight or flight" response. There are three types of cells present in the SNS: chromaffin cells (adrenal medullary cells), small intensely fluorescent cells, and sympathetic neurons (known

as neuroblasts during embryogenesis). The migrating neural crest cells are influenced by the molecular guidance given by various external factors and ligands acting on the receptors present on the surface of the neural crest cells. The morphology, differentiation, and derivatives of the neural crest cells are also mediated by local tissue interactions. Genetically regulated cell-autonomous factors or exposure to environmental factors result in disturbances of differentiation, which then give rise to uncontrolled cell cycle or ectopic tissue formation. Disturbances in neural crest cell regulation are involved in different serious diseases such as neuroblastoma (*Takahashi et al., 2013; Etchevers et al., 2006*).

## 1.10 Genetics of neuroblastoma

Neuroblastoma may be considered a malignant manifestation of aberrant sympathetic nervous system development. Until recently, however, little was known about the genetic basis of this disease. As has been shown for many human cancers, a subgroup of cases display autosomal dominant inheritance (*Knudson and Strong, 1972*). Mossé and colleagues reported that activating mutations in the tyrosine kinase domain of the ana-plastic lymphoma kinase (*ALK*) oncogene account for most cases of hereditary neuroblastoma (*Mossé etal., 2008*). These germline mutations

encode for single-base substitutions in key regions of the kinase domain and result in constitutive activation of the kinase and a premalignant state. Mutations resulting in oncogene activation are also somatically acquired in 5 to 15% of neuroblastomas (*Mossé et al., 2008; Chen et al., 2008*). In humans, *ALK* is located on chromosome 2p23 and the gene encodes for a single-chain transmembrane protein. ALK is a member of the insulin receptor (IR) superfamily of receptor tyrosine kinases, which shows homology with the leukocyte tyrosine kinase, the insulin-like growth factor-1 receptor kinase and the IR kinase. The mutated/amplified full-length ALK leads to cell growth and survival by the activation of the JAK–STAT, PI3K–AKT or RAS–MAPK pathways (*Azarova et al., 2011*). Children with either sporadic or familial neuroblastoma in conjunction with congenital central hypoventilation syndrome, Hirschsprung's disease, or both usually have loss-of-function mutations in the homeobox gene *PHOX2B (Mossé et al., 2004; Trochet et al., 2004)*. Thus, genetic testing for mutations in *ALK* and *PHOX2B* should be considered whenever a patient has a family history of neuroblastoma or has other clinical conditions that are strongly suggestive of a highly penetrant transmissible mutation, such as bilateral primary tumors of the adrenal glands.

Such testing is currently available to practitioners (www.ncbi.nlm.nih.gov/sites/GeneTests).

Although *ALK* and *PHOX2B* mutations account for the majority of familial cases of neuroblastoma, additional familial genes may still be discovered.In sporadic neuroblastoma cases, malignant transformation probably arises from the interaction of common DNA variants in which each individual variation has a relatively modest effect on susceptibility. A genomewide association study of neuroblastoma is currently under way, under the auspices of the Children's Oncology Group (COG). To date, the study has shown that alleles with common single-nucleotide-polymorphism variations within the putative genes *FLJ22536* at chromosome band 6p22.3 and *BARD1* (BRCA1-associated RING domain 1) at 2q35 are significantly enriched among patients in whom neuroblastoma has developed as compared with controls (*Capasso et al., 2009; Corallo et al., 2016*). In addition, the study has also shown that a relatively common copy-number variation at 1q21 is associated with the development of neuroblastoma (*Diskin et al., 2009*). Tumor-derived genomic information has been used since the 1980s to predict the course of newly diagnosed neuroblastomas, with the discovery that the *MYCN* oncogene is the target of the extremely high-level amplifications at chromosome band 2p24 observed in about 20% of neuroblastoma cases (*Zhu and Thomas Look, 2016*). MYCN belongs to a family of oncogenes called Myc oncogene-family members, whose most studied member is the MYC oncogene and include also MYCL oncogene.

They are transcription factors, which activate, together with their dimerization partner Max, gene expression of a number of genes in an E-box dependent manner. MYCN and MYC have very similar molecular functions (*Bonnet et al., 1997*). The Myc oncogene-family members are involved in cell growth through protein synthesis, transcriptional regulation of ribosomal RNA processing, cell adhesion and tumor invasion. A real breakthrough in the search for genomic alterations that impact on NB aggressiveness comes from the recent observation of telomerase reverse transcriptase (TERT) activation by genetic rearrangements in high-risk NB. By whole-genomic sequencing of 59 NB cases the authors discovered recurrent genetic rearrangements in the chromosomal region 5p15.33 proximal of TERT. Rearrangements of this region took place only in high-risk Neuroblastoma (*Peifer et al., 2015*). Taken together, these observations suggest that this developmental childhood cancer is influenced by common DNA variations, facilitating the development of a putative genetic model for this disease.

# 2 AIM

Some advanced tumors often exhibit reduced expression of lamin A/C, even though the mechanisms underlying this downregulation are not yet completely deciphered. Lamins support the nuclear envelope and provide anchorage sites for chromatin; they are also involved in DNA synthesis, transcription, and apoptosis. The involvement of the lamins in the processes of tumor progression, and of subsequent metastasis, may be related to an increased nuclear deformability, which facilitates transit of cells through narrow constrictions such as capillaries. Moreover, lamins can interact with adhesion proteins, such as e-cadherins and nesprins, and could therefore be involved in the process of metastases formation. The aim of my PhD project was to study the possible mechanisms by which Lamin A/C is downregulated in tumors of neural origin and in particular in neuroblastoma. Based on our previous data from my laboratory showing that LMNA gene knock-down inhibits differentiation in a cellular model of neuroblastoma and promote tumor progression by increasing cell motility and invasion *in vitro* and tumorigenicity *in vivo*, I investigated different mechanisms of regulation of the Lamin A/C

expression. As experimental model, I used the SH-SY5Y and LAN-5 human neuroblastoma cell lines expressing different level of LMNA gene. To date I studied the transcriptional regulation of LMNA in terms of histone modifications and TFs recruitment in LAN5 cell line compared to SHSY5Y. Moreover, I also studied the mechanism of regulation of the protein mediated by microRNAs.

# 3 RESULTS

**Lamin A/C expression in neuroblastoma samples and established cell lines.** We have first analyzed by real time PCR the expression of LMNA gene in a cohort of 30 patients with neuroblastoma at different disease stage. We observed a very high heterogeneity with few tumors expressing LMNA gene and many others in which LMNA was low or absent (Fig. 1). Based on these observation we choose for this study two neuroblastoma cell lines showing very different Lamin A/C expression levels. The Western Blotting reported in figure 2A shows the expression levels of Lamin A/C in five different neuroblastoma cell lines. We focused on the LAN-5 cells that do not express Lamin A/C and on the SH-SY5Y cells that instead express Lamin A/C at the highest level.

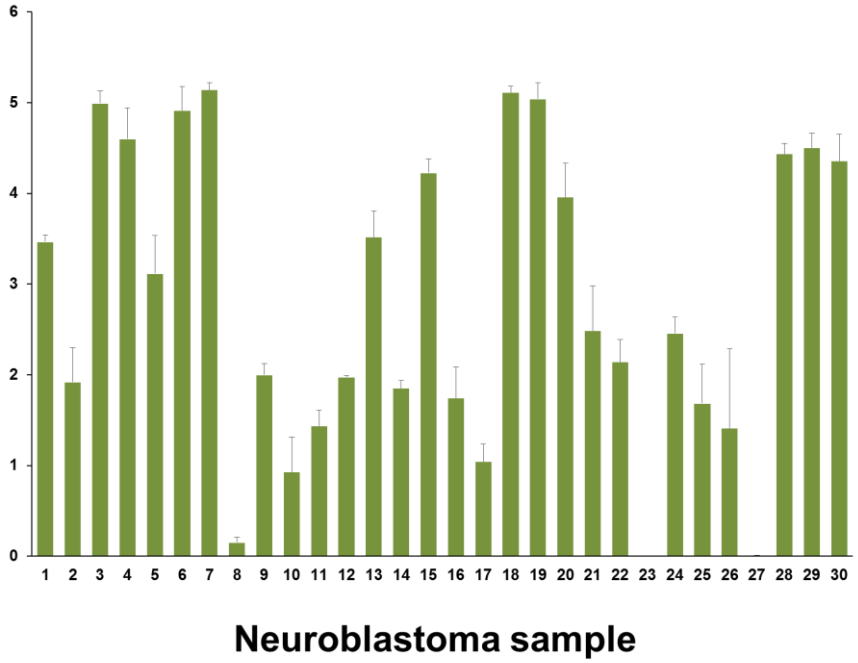
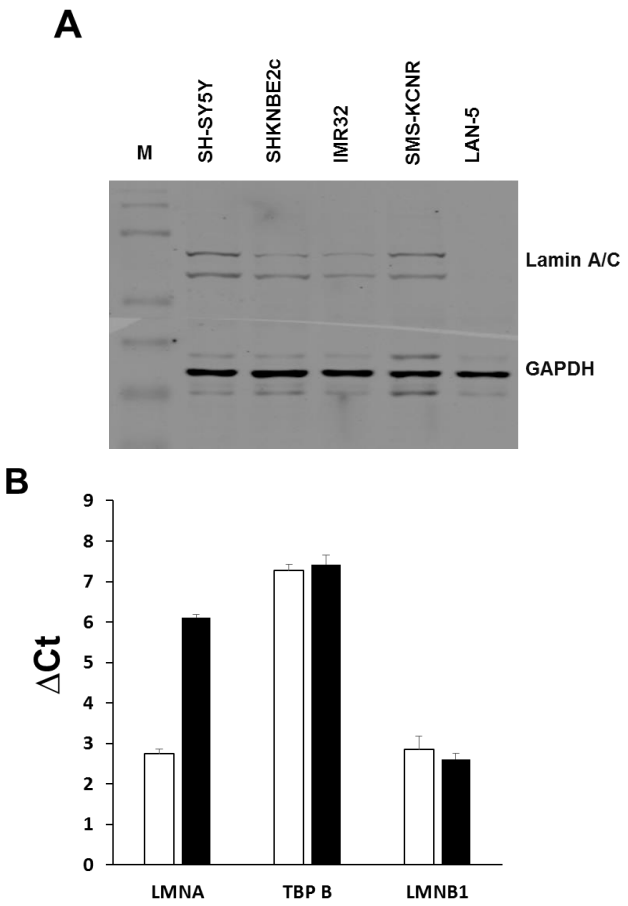



**Fig. 1. LMNA expression in neuroblastoma human biopsies**. qRT-PCR analysis of the LMNA genes in 30 NB human biopsies. Data (mean + SD [n = 3]) are reported as the ΔCt values normalized against the endogenous control. The ΔCt values are inversely correlated with the amount of the gene present in the sample.

The different expression of Lamin A/C protein between the two cell lines was also evident studying the mRNA transcripts. In figure 2B are shown the level of expression of LMNA transcript in SH-SY5Y and LAN-5 cells as evaluated by qRT-PCR. A difference of more than 50% is observed between the two lines; while the levels of other two genes, the housekeeping TBP B and the structural LMNB1, are comparable between the two cell lines.


A
M
SH-SY5Y
SHKNBE2c
IMR32
SMS-KCNR
LAN-5
Lamin A/C
GAPDH
B
ΔCt
0
1
2
3
4
5
6
7
8
9
LMNA
TBP B
LMNB1

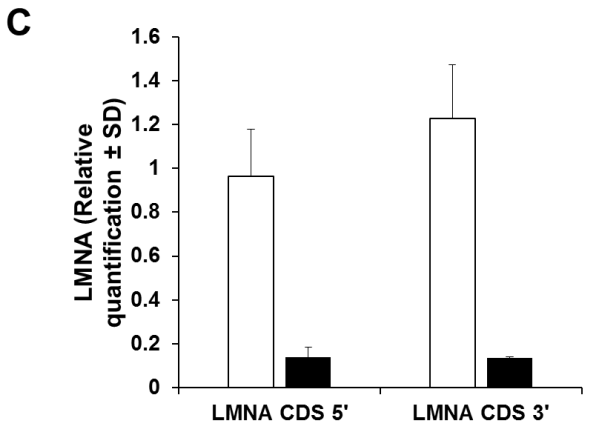



**Fig. 2. Lamin A/C protein expression in NB cell lines**. **A)** Representative blots of the Lamin A/C proteins in the indicated NB cell lines. GAPDH expression was used to normalize protein loading. The experiment was repeated three times with similar results. M, molecular weight markers. **B)** qRT-PCR analysis of the LMNA gene in LAN-5 cells (black) and SH-SY5Y cells (white); as control in the figure is also shown the expression of other two genes, the housekeeping TBP B and the structural LMNB1 genes. Data (mean + SD [n = 3]) are reported as the ΔCt values normalized against the endogenous control. The ΔCt values are inversely correlated with the amount of the gene present in the sample. **C)** qRT-PCR analysis of the not-spliced nascent transcripts of LMNA gene in LAN-5 cells (black) and SH-SY5Y cells (white). The data are reported as the level of mRNA relative to the endogenous control and are the mean + SD [n = 3].

However, mature mRNA is subjected to stabilization processes that could mask the magnitude of the different transcriptional rate of Lamin A/C in SH-SY5Y and LAN-5 cells. Therefore, in order to measure more accurately the transcriptional rate of the LMNA gene we also analyzed the nascent, chromatin-associated, transcript of LMNA gene in both cell lines. After extraction of the nuclear, not spliced, chromatin-associated RNA from both cell lines, the

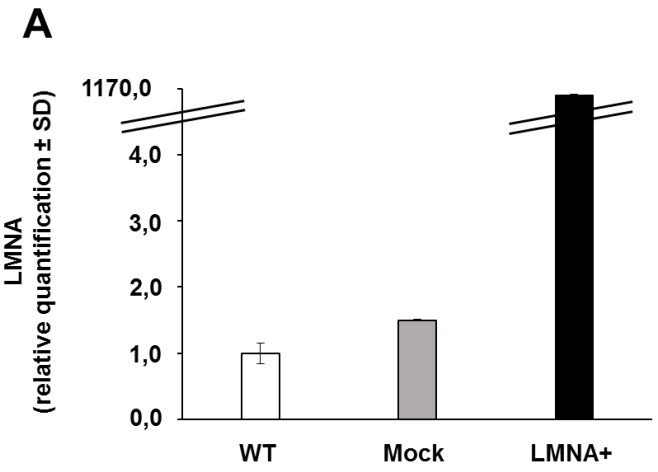
level of LMNA nascent transcript was analyzed using two primer sets specific for the 5' and 3' of the coding region of the gene (Fig. 2C). Specifically, the primers were designed to amplify regions lying across splicing junctions. (Contamination from genomic DNA are excluded by running qRT-PCR reactions with non-reverse transcribed samples [RT-minus samples]).

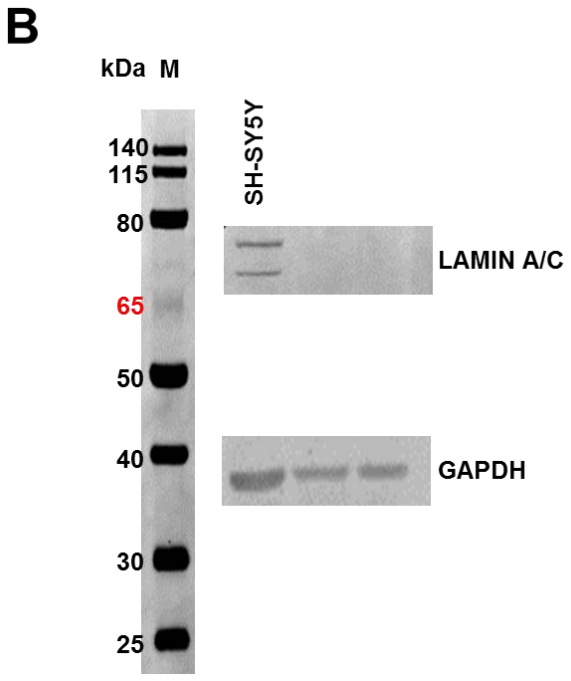
From this analysis we can conclude that the strong reduction of Lamin A/C protein level in LAN-5 cell line compared to SH-SY5Y is to be ascribed to a different transcriptional rate between the two cell lines, apparently due to a transcriptional repression of Lamin A/C in LAN-5 cells.

**Restoring Lamin A/C protein levels in LAN-5 cells by forced expression of LMNA gene.** We then hypothesized that forcing the expression of LMNA gene in LAN-5 cells, which did not show any detectable level of the Lamin A/C protein, could be possible to restore a phenotype similar to that of the SH-SY5Y Lamin A/C expressing cells.

We performed an infection of the LAN-5 cells using a lentiviral vector expressing LMNA gene (EX-Z3407-Lv105 GeneCopeia). The analysis of the gene expression, performed after 48 hours from cell infection, shows a strong over-expression of the LMNA gene already after 48 hours from cell infection (about 1200 folds; Fig. 3A). By contrast, the analysis of protein expression

evaluated by western blot analysis, immunofluorescence and flow cytometry, showed that Lamin A/C was still completely undetectable (Fig. 3B, C and D). Increasing times of observation (up to 14 days after infection) under antibiotic selection did not modify the expression of Lamin A/C. Hence, it is likely that post-transcriptional mechanisms may be involved in the down regulation of Lamin A/C protein in neuroblastoma LAN-5 cells.


A
LMNA
(relative quantification ± SD)
1170,0
4,0
3,0
2,0
1,0
0,0
WT
Mock
LMNA+


B
kDa M
140
115
80
65
50
40
30
25
SH-SY5Y
LAMIN A/C
GAPDH

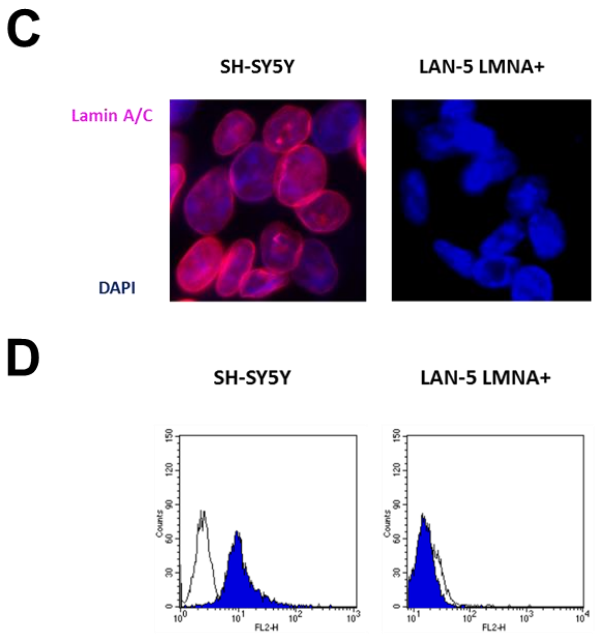



**Fig. 3. Forced LMNA gene expression in LAN-5 cells**. **A)** The levels of LMNA mRNA in LAN-5 WT (white), Mock (grey) and LMNA+ (black) cells. The data are reported as the level of mRNA relative to the respective WT cells and are the mean + SD [n = 3]. **B)** Representative blots of the Lamin A/C protein in LAN-5 Mock and LMNA+ cells. SH-SY5Y cells were used as control for Lamin A/C expression. GAPDH expression was used to normalize protein loading. The experiment was repeated three times showing similar results. M, molecular weight markers. **C)** Representative fluorescent images of SH-SY5Y and LAN-5 LMNA+ cells. Red, Lamin A/C immunostaining; blue, Hoechst. **D)** FACS analysis of the Lamin A/C fluorescence distribution in SH-SY5Y cells and LAN-5 LMNA+ cells. The experiment was repeated three times showing similar results.

**Putative miRNAs targeting LMNA transcripts.** We therefore investigated whether the decreased level of expression of Lamin A/C in LAN-5 could be ascribed to a regulation by miRNAs. To test this hypothesis we performed an *in silico* analysis utilizing the

known databases miRWalk to identify putative miRNAs which can target LMNA gene. In the table 1. are reported the predicted miRNAs ordered by statistical significance. The table specified also the length of each miRNA and the targeting region. We validated the results *in silico* in our cellular models by performing a miRNA expression profiling in LAN-5 and SH-SY5Y cells using TaqMan Human MicroRNA Arrays. A total of 768 miRNAs, present in the array, were analyzed in each cell line. The distribution of the expressed miRNAs is shown in a Venn diagram where a total of 417 (66 specific and 351 common) and of 395 (44 specific and 351 common) miRNAs were found expressed in LAN-5 and SH-SY5Y cells, respectively (Fig. 4). We found 359 and 337 miRNAs not expressed in SH-SY5Y and LAN-5 cells, respectively (293 not expressed at all in both cell lines). We identified a set of 202 out of the 351 common miRNAs differentially expressed at least 2-fold change between the two cell lines (99 in the LAN-5 and 103 in the SH-SY5Y cells); whereas 149 miRNAs were filtered out by the threshold applied. We then identified from the literature the most important genes codifying for proteins involved in the maturation pathway of Lamin A/C (such as ICMT1, ZMPSTE24) or structural nuclear proteins associated to Lamin A/C in forming the nuclear lamina. Using the Miranda tool we analyzed the miRNAs predicted to target the RNA sequence of each of these genes comparing the miRNAs

resulted from this analysis with those derived from the experimental PCR array and filtering in those miRNAs which were upregulated in LAN-5 cells. The table 2 shows only the miRNAs up regulated in the LAN-5 cells which do not express Lamin A/C. We focused on the unique miRNA predicted with a very significant p-value to target LMNA gene, hsa-miR-539-5p.

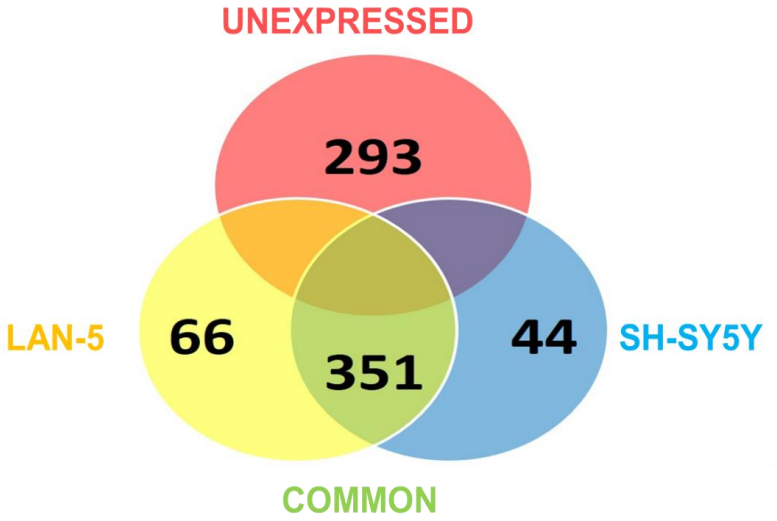



**Fig. 4. Functional analysis of miRNA target genes in LAN-5 and SH-SY5Y cell lines.** Venn diagram of expressed miRNAs in SH-SY5Y and LAN-5 cells. The number represents the total miRNAs expressed in each line.

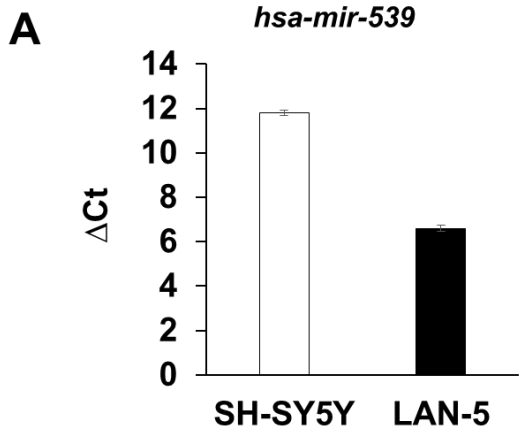
**Table 1. Predicted miRNAs by miRWalk database targeting LMNA gene (NM_170707).**

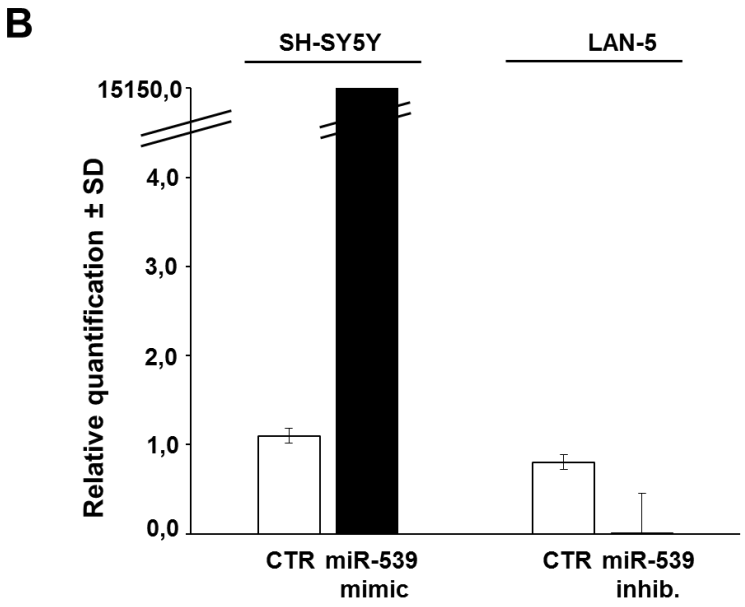
| MicroRNA | Seed length | Start | Position | End | Region | Pvalue |
|---|---|---|---|---|---|---|
| hsa-miR-485-5p | 10 | 2719 | 2 | 2710 | 3 UTR | 0.0010 |
| hsa-miR-539 | 10 | 2490 | 1 | 2481 | 3 UTR | 0.0010 |
| hsa-miR-671-5p | 10 | 2553 | 1 | 2544 | 3 UTR | 0.0010 |
| hsa-miR-1200 | 10 | 2062 | 1 | 2053 | CDS | 0.0019 |
| hsa-let-7e | 10 | 2190 | 2 | 2181 | CDS | 0.0019 |
| hsa-miR-1202 | 10 | 1191 | 2 | 1182 | CDS | 0.0019 |
| hsa-miR-760 | 10 | 2221 | 2 | 2212 | CDS | 0.0019 |
| hsa-miR-1322 | 10 | 2240 | 2 | 2231 | CDS | 0.0019 |
| hsa-miR-138 | 8 | 73 | 2 | 66 | 5 UTR | 0.0038 |
| hsa-miR-615-5p | 8 | 216 | 2 | 209 | 5 UTR | 0.0038 |
| hsa-miR-657 | 8 | 245 | 1 | 238 | 5 UTR | 0.0038 |
| hsa-miR-486-3p | 8 | 223 | 1 | 216 | 5 UTR | 0.0038 |
| hsa-miR-744 | 8 | 199 | 2 | 192 | 5 UTR | 0.0038 |
| hsa-miR-138 | 8 | 73 | 2 | 66 | 5 UTR | 0.0038 |
| hsa-miR-9-5p | 9 | 2982 | 2 | 2974 | 3 UTR | 0.0038 |
| hsa-miR-9-3 | 9 | 2544 | 1 | 2536 | 3 UTR | 0.0038 |
| hsa-miR-576-3p | 9 | 2528 | 2 | 2520 | 3 UTR | 0.0038 |
| hsa-miR-1260 | 9 | 2931 | 2 | 2923 | 3 UTR | 0.0038 |
| hsa-miR-92a-2-5p | 9 | 2836 | 1 | 2828 | 3 UTR | 0.0038 |
| hsa-miR-9-3 | 9 | 2982 | 2 | 2974 | 3 UTR | 0.0038 |
| hsa-miR-9-1 | 9 | 2544 | 1 | 2536 | 3 UTR | 0.0038 |
| hsa-miR-642 | 9 | 3052 | 2 | 3044 | 3 UTR | 0.0038 |
| hsa-miR-9-1 | 9 | 2982 | 2 | 2974 | 3 UTR | 0.0038 |
| hsa-miR-1182 | 9 | 524 | 2 | 516 | CDS | 0.0076 |

**Table 2. Upregulated miRNAs in LAN-5 cells involved in the Lamin functions.**

| | ΔCt | | |
|---|---|---|---|
| **hsa-miR** | **LAN-5** | **SH-SY5Y** | **Target** |
| hsa-miR-539 | 1,8 | 7,8 | LMNA |
| hsa-miR-543 | 5,5 | 27,2 | LAP2 |
| hsa-miR-410 | 4,5 | 11,2 | LMNB1 |
| hsa-miR-224 | 10,2 | 15,0 | |
| hsa-miR-329 | 10,3 | 20,1 | |
| hsa-miR-381 | 13,0 | 19,5 | |
| hsa-miR-186 | 1,1 | 2,7 | |
| hsa-miR-204 | 5,5 | 15,5 | |
| hsa-miR-134 | 5,0 | 12,4 | |
| hsa-miR-381 | 13,0 | 19,5 | MAN1 |
| hsa-miR-204 | 5,5 | 15,5 | |
| hsa-miR-134 | 5,0 | 12,4 | |
| hsa-miR-329 | 10,3 | 20,1 | |
| hsa-miR-410 | 4,5 | 11,2 | |
| hsa-miR-495 | 4,6 | 10,5 | |
| hsa-miR-873 | 12,2 | 15,4 | |
| hsa-miR-381 | 13,0 | 19,5 | ZMPSTE24 |
| hsa-miR-379 | 4,6 | 10,3 | |
| hsa-miR-495 | 4,6 | 10,5 | |
| hsa-miR-488 | 10,8 | 12,5 | |
| hsa-miR-182 | 9,8 | 16,2 | |
| hsa-miR-224 | 10,2 | 15,0 | |
| hsa-miR-433 | 3,8 | 19,5 | |
| hsa-miR-494 | 4,6 | 11,7 | |
| hsa-miR-365 | 7,3 | 9,8 | |
| hsa-miR-488 | 10,8 | 12,5 | ICMT1 |
| hsa-miR-495 | 4,6 | 10,5 | |
| hsa-miR-410 | 4,5 | 11,2 | |
| hsa-miR-186 | 1,1 | 2,7 | |
| hsa-miR-410 | 4,5 | 11,2 | CDK1 |
| hsa-miR-495 | 4,6 | 10,5 | |
| hsa-miR-329 | 10,3 | 20,1 | |
| hsa-miR-143 | 8,0 | 4,8 | |
| hsa-miR-543 | 5,5 | 27,2 | |
| hsa-miR-181a | 7,2 | 5,7 | |
| hsa-miR-181c | 16,1 | 12,6 | |
| hsa-miR-146a | 10,7 | 9,81 | |
| hsa-miR-146b-5p | 16,9 | 14,48 | |
| hsa-miR-374a | 5,1 | 3,9 | PKC alpha |
| hsa-miR-374b | 3,6 | 2,9 | |
| hsa-miR-873 | 12,2 | 15,4 | |
| hsa-miR-186 | 1,1 | 2,7 | SUMO1 |
| hsa-miR-495 | 4,6 | 10,5 | |
| hsa-miR-133a | 4,9 | 8,7 | |
| hsa-miR-133b | 11,6 | 15,2 | |

**Mimicking or inhibiting hsa-miR-539-5p in neuroblastoma cells.** By real time PCR we first verified the expression levels of the hsa-miR-539-5p in both SH-SY5Y and LAN-5 cell lines and the result confirms that this miRNA is really overexpressed in LAN-5 cells by about 50% (Fig. 5A). To investigate whether hsa-miR-539-5p could target LMNA gene we overexpressed or inhibited this miRNA in SH-SY5Y and LAN-5 cells, respectively. The transfection of SH-SY5Y with the miR-539 mimic resulted in approximately 15000-fold increased expression of the miRNA, while the transfection of LAN-5 with the miR-539 inhibitor resulted in a total repression of the miRNA expression (Fig. 5B). However, the expression of the protein was not affected by these treatments in both cell lines as shown in the western blot analysis (Fig. 5C). The results obtained allowed us to exclude miR-539-5p as a possible regulator of the expression of Lamin A/C.




B
SH-SY5Y
LAN-5
15150,0
4,0
3,0
2,0
1,0
0,0
Relative quantification ± SD
CTR
miR-539 mimic
CTR
miR-539 inhib.

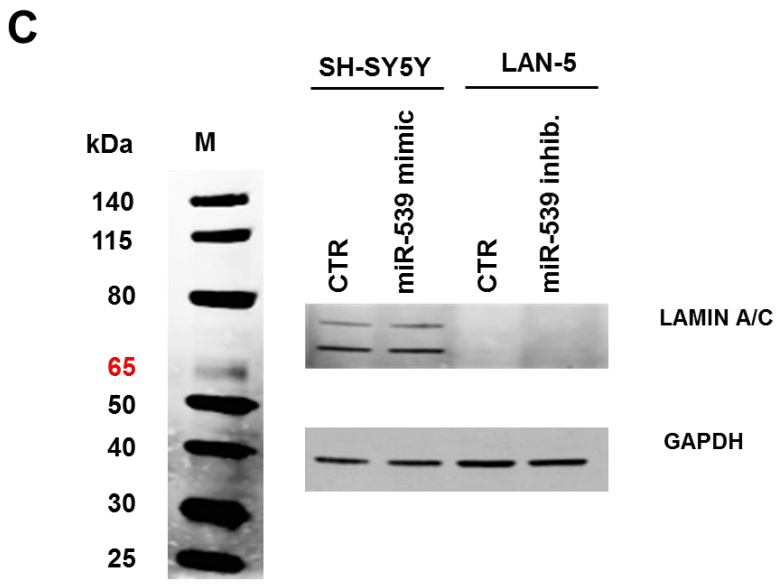

C
SH-SY5Y
LAN-5
kDa
M
140
115
80
65
50
40
30
25
CTR
miR-539 mimic
CTR
miR-539 inhib.
LAMIN A/C
GAPDH

**Fig. 5. Modulation of hsa-miR-539-5p. A)** The levels of hsa-miR-539-5p in SH-SY5Y cells (white) and in LAN-5 cells (black). The data are reported as the ΔCt values normalized against the endogenous control. The ΔCt values are inversely correlated with the amount of the gene present in the sample. **B)** The levels of the hsa-mir-539-5p in mimic negative control (white) and miRNA mimic (black) cells at 24 after transfection in SH-SY5Y cells; and levels of the hsa-mir-539-5p in inhibitor negative control (white) and miRNA inhibitor (black) cells at 24 after transfection in LAN-5 cells. The data are reported as the level of miRNAs relative to the respective miRNAs negative control cells and are the mean + SD [n = 3]. **C)** Representative western blots of the indicated proteins in mimic negative control (CTR) and miR-539-5p mimic cells at 24 after transfection in SH-SY5Y cells and in inhibitor negative control (CTR) and mir-539-5p inhibitor at 24 after transfection in LAN-5 cells. GAPDH expression was used to normalise protein loading. The experiment was repeated three times with similar results. M, molecular weight markers.

**Study of chromatin organization on LMNA promoter.** The ChIP technique is used to study interactions between specific proteins (e.g. transcription factors, transcriptional co-regulators and/or histones) and genomic DNA region (e.g. promoters, enhancers or coding regions of specific genes). Post translational modifications of histones represent an epigenetic mechanism of regulation of gene expression, indeed different combinations of histone modifications at promoters and enhancers lead to specific functional outcomes. We carried out ChIP experiments to analyze the enrichment of specific post-translational histone modifications on LMNA promoter, namely trimethylation of lysine 4 oh histone H3 (H3K4me3), and trimethylation of lysine 27 (H3K27me3) associated with transcriptional activation and transcriptional

repression, respectively. The enrichment of these two histone marks was measured both in SH-SY5Y and LAN-5 cells at two different regions of LMNA promoter, one proximal to the transcription start site (TSS) and a second one upstream of TSS. The LMNA promoter showed a similar chromatin landscape in the two cell lines, specifically a strong H3K4me3 enrichment, whereas no significant enrichment for H3K27me3 were observed, indicating that LMNA promoter display, both in SH-SY5Y and LAN-5 cells, the same open chromatin organization, permissive for transcriptional activation (Fig. 6 A and B).

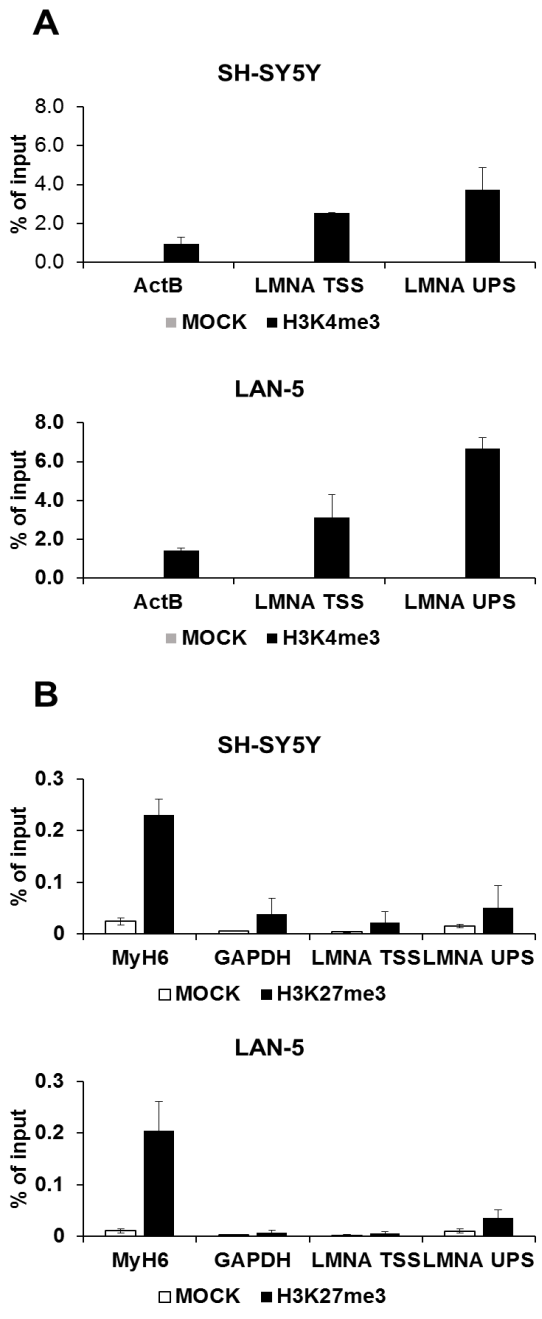

A
SH-SY5Y
% of input
8.0
6.0
4.0
2.0
0.0
ActB
LMNA TSS
LMNA UPS
MOCK
H3K4me3
LAN-5
% of input
8.0
6.0
4.0
2.0
0.0
ActB
LMNA TSS
LMNA UPS
MOCK
H3K4me3
B
SH-SY5Y
% of input
0.3
0.2
0.1
0
MyH6
GAPDH
LMNA TSS
LMNA UPS
MOCK
H3K27me3
LAN-5
% of input
0.3
0.2
0.1
0
MyH6
GAPDH
LMNA TSS
LMNA UPS
MOCK
H3K27me3

**Fig. 6. ChIP analysis of histone modifications on LMNA promoter**. **A)** ChIP-qPCR analysis of H3K4me3 at two different regions of the LMNA promoter and at ActB promoter in SH-SY5Y and LAN-5 cells. **B)** ChIP-qPCR analysis of H3K27me3 at two different regions of the LMNA promoter and at GAPDH, MyH6 promoters in SH-SY5Y and LAN-5 cells. The data have been normalized to input. Error bars, of three independent experiments.

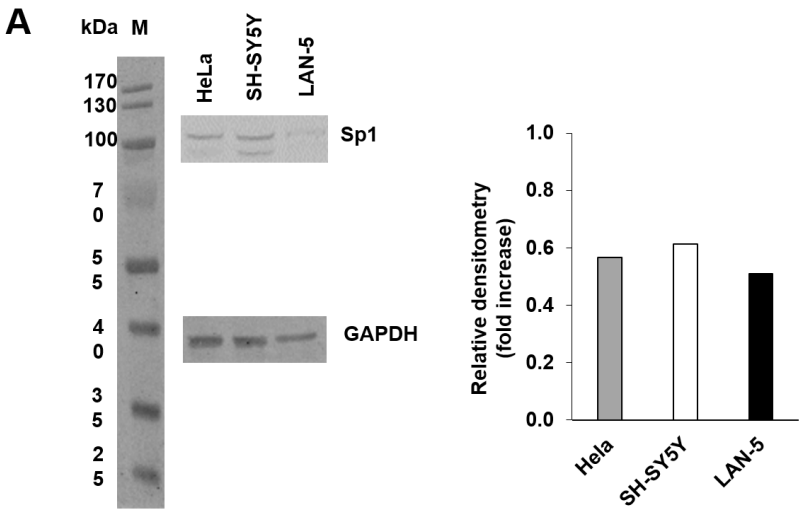
**Study of Sp1 recruitment on LMNA promoter.** The similar enrichment pattern of histone modifications associated with gene activation or repression (specifically H3K4me3 and H3K27me3) on LMNA promoter in SH-SY5Y and LAN-5 cells does not explain the different transcription rate observed in these two cell lines.

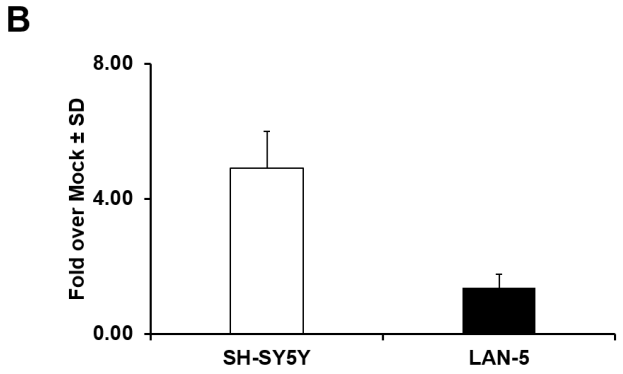
We decided to analyze the recruitment of Sp1 transcription factor, a member of the Sp transcription factor family, on LMNA promoter. First, we analyzed the constitutive expression of Sp1 protein in SH-SY5Y and LAN5 cells. The western blot analysis in figure 7A shows that Sp1 is expressed at similar levels in the two cell lines. ChIP experiments were performed on chromatin derived from both cell lines and demonstrate a lower recruitment of Sp1 on the LMNA promoter in LAN-5 cells (Fig. 7B), thus correlating to the reduced transcription of LMNA in these cells. Presumably, the reduced recruitment of Sp1 transcription factor determines a lower recruitment of basal transcriptional machinery on LMNA promoter in LAN-5 cells.

This latter hypothesis was confirmed by analyzing the level of trimethylation of Lys36 of histone H3 along the coding region of LMNA gene; this histone modification is catalyzed by a histone methyltransferase travelling with RNA polymerase II during elongation, thus representing a mark of of actively transcribed genes. The chromatin immunoprecipitation experiments demonstrate that this mark is lower in LAN-5 cells, thus correlating to the reduced transcription rate of LMNA gene in this cell line (Fig. 7C).

Collectively, we found that, despite of a permissive chromatin configuration, the transcription factor Sp1 and, consequently, the Polymerase II transcriptional machinery, are not recruited on LMNA promoter of LAN-5 cells.

Localized DNA hypermethylation of promoter regions leading to transcriptional repression were found to be a common feature of many cancers. Ongoing experiments aim at analyzing DNA methylation of LMNA locus in SH-SY5Y and LAN-5 cells in order to verify whether a different pattern of DNA methylation could be responsible for the different regulation of LMNA expression.


A
kDa M
HeLa
SH-SY5Y
LAN-5
170
130
100
70
55
40
35
25
Sp1
GAPDH
Relative densitometry (fold increase)
1.0
0.8
0.6
0.4
0.2
0.0
Hela
SH-SY5Y
LAN-5


B
Fold over Mock ± SD
8.00
4.00
0.00
SH-SY5Y
LAN-5

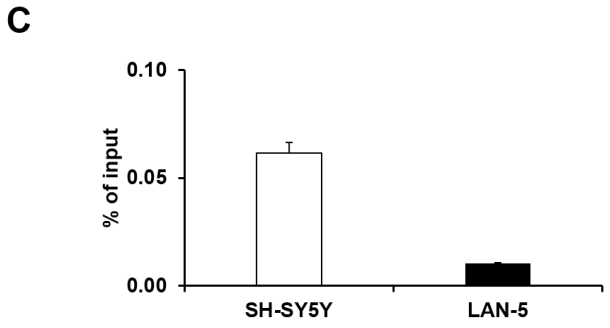

C
% of input
0.10
0.05
0.00
SH-SY5Y
LAN-5

**Fig. 7. Study of interaction of LMNA promoter with the Sp1 TF by ChIP. A)** Western blot analysis and relative densitometry of Sp1 protein in HeLa, SH-SY5Y and LAN-5 cells. GAPDH expression was used to normalize protein loading. The experiment was repeated three times showing similar results. M, molecular weight markers. **B)** ChIP-qPCR analysis of Sp1 binding to LMNA promoter in SH-SY5Y and LAN-5 cells. The data shows the fold over Mock. Error bars, of three independent experiments. **C)** ChIP analysis of H3K36me3 modifications on LMNA coding sequence in SH-SY5Y and LAN-5 cells. The data have been normalized to input. Error bars, of three independent experiments.

# 4 DISCUSSION

A-type lamins are essential components of the nuclear lamina. Together with B-type lamins they form the network located in the inner side of the nuclear membrane. Lamin A/C have been suggested as a biomarker for various types of cancer (*Foster et al., 2010*). Lamin A/C are absent in embryonic stem cells and some adult stem cell types but are expressed in the majority of cell types in adults, thereby suggesting that their expression is an indicator of differentiation (*Lin et al., 1997*). Mutation in LMNA gene causes the Emery-Dreifuss muscular dystrophy (EDMD) (*Bonne et al., 1999*), from 1999 more than 450 diseases have been associated with mutations of LMNA gene, and are collectively referred to the term *Laminopathies*. These disorders include Hutchiston-Gilford progeria syndrome (HGPS), dilated cardiomyopathy with conduction defect (DCM-CD), the familiar partial dystrophy Dunningan (FPLD), the Charcot-Marie Tooth (CMTS) and many others. The most studied among these diseases is HGPS, in this case a mutation leading to aberrant splicing of prelamin A transcripts, the protein Lamin A/C lacks 150 nucleotides (AΔ50) and leads to the production of progerin, a major, internally truncated prelamin A, which is processing-incompetent and

accumulates in the nuclei of the cells, exerting multiple toxic effects. Other aberrant transcripts as prelamin AΔ35 and prelamin AΔ90 have been involved in the pathophysiology of specific progeroid syndromes (*Hisama et al. 2011*). As concern the relationship between lamins and cancer even though contrasting results are present in literature in general, most malignant tumors present decreased levels of Lamin A/C. Loss of lamin A/C expression has been reported for colon cancer (*Willis et al., 2008*), lung cancer (*Machiels et al., 1995*), prostate cancer (*Debes et al., 2005*), gastric cancer, breast cancer (*Callinice et al., 2011*), leukemia and lymphoma (*Agrelo et al., 2005*). A down regulation of Lamin A/C could result in increased nuclear deformability thereby facilitating transit of cells through capillaries and favoring cell invasion **(***Foran et al., 2006*). During my PhD I studied some of the mechanisms possibly underlying the reduced expression of Lamin A/C protein in a NB cellular model, the LAN-5 cells. In the LAN-5 cells there is a complete loss of Lamin A/C protein levels compared to the control SH-SY5Y cells, which instead presented high levels of the protein. The decreased protein levels in LAN-5 cells were accompanied by a reduced transcription rate of the LMNA gene (about 3-folds) with respect to the SH-SY5Y control cells. Moreover, the analysis of the nuclear, not spliced, chromatin-associated RNA confirm the reduced transcriptional rate in the LAN-5 cells. Our first hypothesis was that a transcriptional

regulation mechanism is responsible of the downregulation of LMNA gene. In order to study whether an overexpression of LMNA gene could modify the biological behavior in the LAN-5 cells we decided to force the expression of LMNA gene in these cells by using a lentiviral vector carrying an LMNA construct. Even if the transcripts of the LMNA gene were significantly up regulated by about 1200-folds after infection, the protein was still undetectable. Hence, it is likely that not only a transcriptional but also a post-transcriptional mechanism could be involved in the down regulation of Lamin A/C protein in LAN-5 cells. As first mechanism, we investigated the mRNA targeting by microRNAs (miRNAs). miRNAs have a strong influence on gene expression, and they allow simultaneous regulation of multiple components of the signaling network in normal development, differentiation, growth and in human diseases such as cancer (*Jansson et al., 2012*). The *in Silico* analysis I performed using the database miRWalk allowed identifying several putative miRNAs, which could target LMNA gene. The unique miRNA predicted with a very significant p-value to target LMNA gene was the hsa-miR-539. In order to verify the possibility to restore Lamin A/C expression in LAN-5 cells we inhibited hsa-miR-539 in these cells using the mirVana reagents. Simultaneously, in the SH-SY5Y control cells we overexpressed the same miRNA in order to down regulate LMNA gene. The modulation of hsa-miR-539 in both cell

lines did not result in any modification of the Lamin A/C expression thus demonstrating that miR-539 is not involved in the knockdown of the Lamin A/C protein. However, we cannot exclude that other miRNAs targeting other components of nuclear lamina could be involved in the Lamin A/C regulation.

We also studied whether epigenetic mechanisms such as histone modifications of LMNA promoter could be involved. Histone modifications in the cancer landscape are markers of function and chromatin state. Aberrant histone methylation frequently occurs in tumor development and progression. Multiple studies have identified that histone lysine methyltransferases regulate gene transcription through the methylation of histones, which affects cell proliferation and differentiation, cell migration and invasion, and other biological characteristics. Histones have variant lysine sites for different levels of methylation, catalyzed by different lysine methyltransferases, which have numerous effects on human cancers. For example the knockdown of SETDB1 (a specific methyltranspherase for lysine 9 of Histone 3) reduced lung cancer cell growth *in vitro* and *in vivo,* and overexpression of SETDB1 promoted cancer cell invasiveness (*Schultz et al., 2002*). Another example is the methyltrasferase zeste protein-2 (EZH2) specific for lysine 27 of Histone 3 (H3K27). Enhancer of EZH2, as a catalytic component of the polycomb repressive complex 2, catalyzes

histone H3K27 tri-methylation. To date, >300 studies have reported a close correlation between EZH2 and 46 types of human cancer. EZH2 is commonly overexpressed in the majority of common cancers, and high EZH2 expression is a prognostic indicator of poor survival. In breast cancer, downregulation of EZH2 blocks the cell cycle, and suppresses cell growth and survival (*Jansen et al., 2012*). MYND domain-containing protein 3 (SMYD3) is other methyltransferases specific for lysine 4 of histone 3 (H3K4). In colorectal cancer and esophageal squamous cell carcinoma, knockdown of SMYD3 impairs cell proliferation (*Peserico et al., 2015; Dong et al., 2014*). In our models the study of two histone markers, such as H3K4me3 and H3K27me3, on two different region of LMNA promoter, demonstrated that LMNA promoter has the same permissive chromatin organization in both LAN-5 and SH-SY5Y cell lines. This indicates that a transcriptional control mechanism is not mediated by histone modifications.

We further investigated the effect of the Sp transcription factors (TFs) family on the LMNA promoter. We considered the Sp1 TF which is the one recruiting the transcriptional complex at the LMNA promoter. Sp1 was considered a general TF required for transcription of a large number of 'housekeeping genes' (*Black et al., 2001*). However, recently a study has indicated that many of

these housekeeping genes are crucial in tumorigenesis and cancer progression because it can activate and suppress the expression of a number of essential oncogenes and tumor suppressors (*Beishline et al., 2015*). Consequently, Sp1 has been demonstrated to be important in tumor progression, including cell proliferation, angiogenesis, differentiation, apoptosis, migration and invasion, revealing it as an ideal target for cancer treatment (*Sankpal et al., 2011*). ChIP data shows that there is a lower recruitment of Sp1 TF on the LMNA promoter in LAN-5 cells with respect to SH-SY5Y cells. Hence, the transcription of LMNA gene could be reduced by this mechanism.

In conclusion, we demonstrated that the main regulation mechanism involved in the down-regulation of LMNA gene in NB cells is a transcriptional mechanism. We cannot exclude that also a post-translational regulation mechanism could mediate the strong knock-down of Lamin A/C observed in LAN-5 cells. Possibly protein degradation mediated by proteasome could occur. A mechanism that needs to be investigated in detail. The 26S proteasome is an essential protein complex responsible for degrading the majority of cellular proteins in eukaryotes. This mechanism is complex and it is regulated at several levels by multiple mechanisms, ranging from transcriptional control to post-translational modifications of proteasome subunits. An impaired

proteasome system often underlies neurodegenerative diseases and the aging process (*López-Otín et al., 2013*). On the other hand, the rapid growth of cancer cells is often dependent on elevated proteasome activity in many types of tumors such as myeloma and certain solid cancer (*Hoeller et al., 2009; Guo et al., 2016*). For example, it is known that proteasome inhibition results in reduction of cancer progression in cervical cancer cells (*Rastogi et al., 2015*). Moreover, Lamin A/C proteins are target for serine/threonine (SER/THR) kinases that are overexpressed in cancer cells (e.g. p-AKT) (*Naeem et al., 2015*). As matter of fact, p-Akt is viewed as a promising therapeutic target in cancer pathology and therapy: drugs that can inhibit SER/THR kinase activities of p-AKT may also restore Lamin A/C.

Reduction of Lamin A/C in cancer cells may also be an important mechanism producing aneuploidy and chromosomal numerical instability hypothesizing that the loss of Lamin A/C, associated with additional oncogenic mutations, could impair cell growth and trigger tumor progression (*Callinice et al., 2011*). Cells bearing mutations in LMNA gene or lacking Lamin A/C display abnormal nuclear morphology, defective cell cycle kinetics, polyploidy, loss of heterochromatin organization and chromosomal aberration, all of which are hallmark for dedifferentiated cancer cells.

# 5 MATERIALS AND METHODS

## Human NB biopsy RNA extraction and real-time RT-PCR

Total RNA was extracted from thirty frozen biopsies of human newly diagnosed NBs obtained from Department of Pediatrics and Infantile Neuropsychiatry of Sapienza University, Rome using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. RNA was reverse-transcribed, and real-time PCR performed as described above. Institutional written informed consent was obtained from the patient's parents or legal guardians according to the local institutional guidelines.

## Cell line maintenance

The SH-SY5Y NB cell line was purchased from ATCC. These cells were seeded in adherent conditions in the presence of 1:1 mixture of Eagle's Minimum Essential Medium and F12 medium (Gibco) supplemented with 10% FBS (Hyclone), 2 mM L-glutamine, 0.5% non-essential amino acids, 0.5% sodium pyruvate and 1% penicillin and streptomycin. The LAN-5 Neuroblastoma (NB) cell line (a gift from Dr. Doriana Fruci) was grown in RPMI-1640 medium (Gibco) supplemented with 10% FBS (Hyclone), 2

mML-glutamine and 1% penicillin/streptomycin in a fully humidified incubator containing 5% $CO_2$ at 37°C.

## Western blot analysis

SH-SY5Y and LAN-5 NB monolayers were washed twice with 1X PBS and then incubated for 1 min in urea buffer (8M urea, 100mM NaH2PO4 and 10mM Tris pH 8), harvested and briefly sonicated. The proteins were run on a pre-cast polyacrylamide NuPAGE 10% Bis-Tris gel and NuPAGE 4-12% Bis-Tris gel (Life Technologies). The resolved proteins were blotted overnight onto nitrocellulose membranes, then blocked in 1X PBS containing 5% non-fat milk for at least 1 h. The blots were incubated with the following primary anti-human antibodies: monoclonal anti-Lamin A/C (clone JOL2; Chemicon International); monoclonal anti-Sp1 (ab13370, Abcam) monoclonal anti-GAPDH (6C5; Millipore). After washing, the membranes were then incubated for 45 min with the secondary donkey anti-mouse or anti-rabbit antibody IRdye800 (LI-COR). After further washing, membranes were then analysed with a Licor Odyssey Infrared Image System in the 800 nm channel. Blot scan resolution was 150 d.p.i.

## Total RNA preparation

Total RNA was isolated using a Total RNA purification kit (Norgen Biotek, Thorold, ON, Canada). RNA quantity was determined by measuring absorbance at 260 nm using a NanoDrop UV-VIS spectrophotometer. The quality and integrity of each sample was confirmed using a BioAnalyzer 2100 (Agilent RNA 6000 Nanokit); samples with an RNA Integrity Number (RIN) index lower than 8.0 were discarded.

## Real-time RT-PCR analysis

RNA (250 ng) was retro-transcribed with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem) according to the manufacturer's instructions. Equal amount of cDNA was then subjected to real time PCR analysis with an Applied Biosystems 7500HT thermal cycler, using the SensiMix SYBR Kit (Bioline) and the following specific primers at a concentration of 200 nM. Each experiment was performed in triplicate. The expression data were normalized using the Ct values of GAPDH and TBP.

| Primer | Unigene | Forward Sequence | Reverse Sequence |
|---|---|---|---|
| LMNA | Rn.44161 | GAGCAAAGTGCG TGAGGAGT | TCCCCCTCCTTC TTGGTATT |
| GAPDH | Hs.544577 | AGCCACATCGCTC AGACA | GCCCAATACGA CCAAATCC |
| TBP | Hs.590872 | GAACATCATGGA TCAGAACAACA | ATAGGGATTCC GGGAGTCAT |

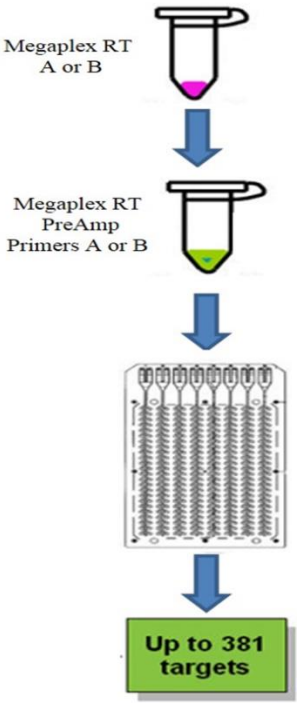
## Cell infection

Proliferating LAN-5 cells were transfected with a plasmid carrying the LMNA construct driven by the CMV promoter (pEZ-Lv105, GeneCopoeia) using Lipofectamine2000 (Life Technologies) according to the manufacturer's instructions. We used 2.5 µg of DNA per sample. LMNA expression was monitored by checking the simultaneous coexpression of the EmGFP reporter gene by fluorescence microscopy.

## PCR Array

RNA was reverse transcribed using TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems). cDNA was preamplified using TaqManPreAmp Master Mix (Applied Biosystems). qRT-PCR was performed with an Applied Biosystem7500HT thermal cycler using TaqMan human microRNA array (TaqMan Human microRNA Array A #4398977 and B v3.0 #442812; Applied Biosystems) according to manufacturer's instructions. The downstream analysis filtered out miRNAs not detected in both cell lines (293), whereas those specifically expressed either in LAN-5 or in SH-SY5Y were considered and reported as cell line-specific. Data were then normalized calculating the ΔCt value for single miRNA against the average of the specific controls for each card according to manufacturer's instructions. Differential expression analysis was

performed according to ΔΔCt method and only RQ ≥ 2 fold-change were considered for further analysis. miRNAs clusters were generated through the DIANA web tool mirPath v2.0 using miRBase MIMAT IDs (Release 21) remapped to the newest human genome assembly (GRCh38) to avoid duplicate entries present in the previous release. Uniquely targets reported in TarBase database v7.0 were included in the clustering, predicted targets were not taken into account. False Discovery Rate (FDR) correction was applied to the original p-value and only clusters with corrected p-values < 0.05 were shown.



## miRNA Assays

Equal amounts of RNA were reverse transcribed with the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions, with a custom 1X RT primer pool (hsa-miR-539-5p ID 001286; has-miR-16ID 000391; U6 snRNA ID 001973). Real time PCR analysis was performed with an Applied Biosystems 7500HT thermal cycler using 20X Individual TaqMan® MicroRNA Assays.

## Hsa-miR-539 inhibitor and mimic

LAN-5 and SH-SY5Y cells were seeded at a density of $3x10^5$ cells in 35 mm dishes. After 24 h, the cells were transfected overnight in the presence of 10% FBS with mirVana miRNA inhibitor, miRNA mimic or the respective negative controls. Lipofectamine2000 (Life Technologies) was used as the transfection reagent, according to the manufacturer's instructions. We transfected the has-miR-539 inhibitor (MH11336, Ambion) and the mimic (MC11336, Ambion) at a final concentration of approximately 30 nM. The mirVana miRNA inhibitor and mimic negative control were used at the same final concentration of 30 nM. Samples were harvested after 24 h from transfection to perform mRNA expression analysis.

## Chromatin Immunoprecipitation (ChIP)

Cells were grown up to 80% of confluence before harvesting. For chromatin crosslinking formaldehyde (Sigma: F8775) was added directly to culture medium to a final concentration of 1% and incubated for 10 minutes at room temperature; then, formaldehyde wasquenched by addingTris-HCl, pH 7.5 (0.125M final concentration) for 5 minutes and cells were washed 3 times with ice-cold PBS. Fixed cells were collectedin cold PBS by scraping and spinned down by centrifugation. Fixed cell pellets can be flash frozen and stored at −80°C or directly used to prepare nuclear extracts.

Nuclear extracts were prepared as follows:

- Lysis of cytoplasmatic membrane by LB1 buffer (50 mMHepes-KOH pH 7.5, 140 mMNaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100). Keep on ice for 10 minutes and spin down the nuclei.
- Wash of nuclei and removal of detergents by LB2 buffer (10 mMTris-HCl, pH 8.0, 200 mMNaCl, 1 mM EDTA, 0.5mM EGTA).Rock gently at room temperature for 10 minutes and spin down the nuclei.
- Lysis of nuclei by LB3 buffer (10 mMTris-HCl, pH 8.0 , 100 mMNaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine)

All lysis buffer were supplemented with protease inhibitors (Protease complete Inhibitor Cocktail - Roche: 04693132001). Nuclear extracts were sonicated using an ultrasonic Bioruptor sonicator to shear chromatin to an average fragment size of 300-700 bp; DNA fragments were extracted starting from a small amount of sonicated chromatin and checked on agarose gel.

The sonicated chromatin was used for immunoprecipitation with 2-5 μg of the antibody of interest previously coupled to protein G (Life Technologies #10004D), overnight with constant rotation at 4°C. The antibodies used for the experiments described in the results are reported below. The immune-complexes were then sequentially washed 6 times with cold washing buffer (50 mMHepes-KOH, 500 mMLiCl, 1 mM EDTA, 1% NP-40, 0.7% Na-Deoxycholate) and once with TE 50mMNaCl, and then extracted with Elution Buffer (10mM Tris-HCl, pH 8.0, 5mM EDTA, 300mM NaCl, 0.5% SDS). Protein-associated DNA fragments were recovered by reversion of crosslinking (65°C overnight) followed by DNA extraction with SPRI cleanup beads (AMPureBeckman #A63881). ChIP-DNA was then quantified by Quant-IT Picogreen dsDNA Assay kit (#P11496) prior to proceeding to qPCR analysis. Primers used for ChIP analysis by qPCR are listed below.

Antibodies list

- Anti-trimethyl-Histone H3 (Lys4) (Anti-H3K4me3) - Abcam ab-8580
- Anti-trimethyl-Histone H3 (Lys27) (Anti-H3K27me3) - Millipore 07-449
- Anti-Sp1 - Abcam 13370
- Anti-trimethyl-Histone H3 (Lys36) (Anti-H3K36me3) - Abcam ab-9050

Human ChIP primers

| **Target Name** | **Primer Name** | **Forward sequence** | **Reverse sequence** |
|---|---|---|---|
| Lamin A/C | LMNA TSS | TCGGACCTGGGAG TCTCTAG | AAGAGTTTGAGCG CTGGAAG |
| Lamin A/C | LMNA UPS | TCCTAGTTCTCCA AAGCCCC | TTTCCCTTCCACCA CCTCTC |
| Lamin A/C | LMNA CDS 5’ | GCGTGAGGAGTTT AAGGAGC | GCAAAGTTATCGG CCTCCAG |
| Actin Beta | ActB | CCCAACACCACAC TCTACCT | CTTGCCGACTTCA GAGCAAC |
| Myosin heavy chain 6 | MyH6 | TTGGGGAACAGAA GGAGACC | CCAGGGAAGGGAT CTGTTGT |
| GAPDH | GAPDH | CCAATCTCAGTCC | CCCTACTTTCTCCC |

| | | | |
|---|---|---|---|
| | | CTTCCCC | CGCTTT |
| GAPDH | GAPDH CDS5' | CACATCGCTCAGA CACCATG | CATACGACTGCAA AGACCCG |
| STAT1 | STAT1 | TGTAGGTGTGAAG CCCAGAG | GGCTTCTCCTAAA CGTGTG |

## Nascent Transcripts

Cells were grown up to 80% of confluence before harvesting. The nuclei were prepared in a lysis buffer (50mM Tris-HCl pH 8.0; 0.2mM EDTA pH 8.0; 1% NP-40; 10% glycerol), and purified by sucrose cushion (1.5mM Hepes pH 7.9; 0.5mM EDTA; 15 mMNaCl; 60 mMKCl;10% glycerol; 0.9 M sucrose). The nuclei were then resuspended in nuclear resuspension buffer (0.5mM EDTA; 20mM Tris-HCl pH 7.5; 75 mMNaCl; 50% glycerol; 100 µg/ml Yeast tRNA), before lysis with nuclear lysis buffer (20 mMHepes pH 7.6; 0.2mM EDTA; 0.3 M NaCl; 1% NP-40; 7.5 mM$MgCl_2$; 1 M Urea; 100 µg/ml Yeast tRNA. All buffers were supplemented withDTT (1mM), PMSF (0.5 mM), spermine (0.15mM) and spermidine(0.5mM).RNA was then isolated using a Total RNA purification kit (NorgenBiotek#48300) or standard Trizol protocol (Invitrogen). RNA concentration was quantified by NanoDrop measurement of the absorbance at 260 nm.0.5 µg of RNA was used for reverse transcription with standard protocol. cDNA was amplified by qPCR with primers specific for pre-

mRNA (designed to amplify either intronic regions, or regions lying across splicing junctions). Contamination from genomic DNA are excluded by running qPCR reactions with non-reverse transcribed samples (RT-minus samples).Primers used for nascent transcripts analysis by qPCR are listed below.

Human nascent transcripts primers

| Target Name | Primer name | Forward sequence | Reverse sequence |
|---|---|---|---|
| Lamin A/C | LMNA CDS 5' | GCGTGAGGAGTTTAAGGAGC | GCAAAGTTATCGGCCTCCAG |
| Lamin A/C | LMNA CDS 3' | AATCTGGTCACCCGCTCCTA | TCCTACCCCTCGATGACCA |
| TBP | TBP CDS | AGTTCTGGGATTGTACCGCA | GTGCCACTCCCTCCCTTAAT |

## Statistical analysis

Student's t test (unpaired, two-tailed) was used for statistical comparison between two groups. If there were more than two groups, we used the one-way ANOVA test.

# 6 REFERENCES

Agrelo R. *et al*. Inactivation of the lamin A/C gene by CpG island promoter hypermethylation in hematologic malignancies, and its association with poor survival in nodal diffuse large B-cell lymphoma. *J Clin Oncol*. Jun 10;23(17):3940-7. Epub 2005 May 2 (2005).

Alsheimer, M., von Glasenapp, E., Hock, R., and Benavente, R. Architecture of the nuclear periphery of rat pachytene spermatocytes: Distribution of nuclear envelope proteins in relation to synaptonemal complex attachment sites. *Mol.Biol. Cell* 10, 1235–1245 (1999).

Andres V. and Gonzalez J.M. Role of A-type lamins in signaling, transcription, and chromatin organization. *J. Cell Biol.187*, 945-957 (2009).

Azarova AM, Gautam G, George RE. Emerging importance of ALK in neuroblastoma. *Semin Cancer Biol* 21: 267–275 (2011).

Barbie M. Machiels, Antoine H. G. Zorenc, Jorike M. Endert, Helma J. H. Kuijpers, Guillaume J. J. M. van Eys, Frans C. S. Ramaekers, and Jos L. V. Broers An Alternative Splicing Product of the Lamin A/C Gene Lacks Exon 10. Vol. 271, No. 16, Issue of April 19, pp. 9249–9253, (1996).

Bartel D. P., (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136(2): 215-233.

Beishline K, Azizkhan-Clifford J. Sp1 and the 'hallmarks of cancer'. *FEBS J.* Jan; 282(2):224-58. (2015)

Belt E.J.T. *et al*. Loss of lamin A/C expression in stage II and III colon cancer is associated with disease recurrence. *Eur. J. Cancer* 47, 1837–1845 (2011).

Bigger, C.B., Melhikova, I.N. & Gardner, P.D. Sp1 and Sp3regulate expression of the neuronal nicotinic acetylcholinereceptor b4 subunit gene. *J. Biol. Chem*. 272, 25976±25982 (1997).

Black AR, Black JD, Azizkhan-Clifford J Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. *J Cell Physiol.* Aug; 188(2):143-60 (2001).

Bonne, G. *et al*. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery–Dreifuss muscular dystrophy. *Nat. Genet*. 21, 285–288(1999).

Bossie C.A. and Sanders M.M. A cDNA from *Drosophila melanogaster* encodes a lamin C-like intermediate filamentprotein. *J. Cell Sci.* 104, 1263–1272 (1993).

Botto N., Vittorini S., Colombo M.G., Biagini A., Paradossi U., Aquaro G., Andreassi M.G. A novel LMNA mutation (R189W) in familial dilated cardiomyopathy: evidence for a 'hot spot' region at exon 3: a case report. *Cardiovasc Ultrasound*. Mar 22;8:9. doi: 10.1186/1476-7120-8-9 (2010).

Brodeur G. M., Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 3(3): 203-216 (2003).

Broers J. L. V., Ramaekers F. C. S., Bonne G., Ben Yaou R., Hutchison C. J. Nuclear Lamins: Laminopathies and Their Role in Premature Ageing, *Physiol Rev Pub* July 2006 Vol. 86 no. 3, 967-1008 DOI: 10.1152/physrev.00047 (2005).

Brown C.A., Lanning R.W., McKinney K.Q., *et al*. Novel and recurrentmutations in lamin A/C in patients with Emery–Dreyfuss musculardystrophy. *Am J Med Genet*;102:359-67 (2001).

Cain N.E., Tapley E.C., McDonald K.L., Cain B.M., Starr D.A. The SUN protein UNC-84 is required only in force-bearing cells to maintain nuclear envelope architecture. *J Cell Biol.* Jul 21; 206(2):163-72 (2014).

Capasso M, Devoto M, Hou C, et al. Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. *Nat Genet*. ;41:718–23. (2009).

Chang W., Worman H.J., Gundersen G.G. Accessorizing and anchoring the LINC complex for multifunctionality. *J Cell Biol*. Jan 5; 208(1):11-22 (2015).

Chen H. *et al*. The nuclear lamina regulates germline stem cell niche organization via modulation of EGFR signaling. *Cell Stem Cell* 13, 73–86 26 (2013).

Chen Y., Takita J., Choi Y.L., et al. Oncogenic mutations of ALK kinase in neuroblastoma. *Nature*. 455:971–4 (2008).

Clarke S. Protein isoprenylation and methylation at carboxyl-terminalcysteine residues. *Annu. Rev. Biochem*. 61, 355-386 (1992).

Corallo S. Candiani S., Ori M., Aveic S., Tonini G.P. The zebrafish as a model for studying neuroblastoma. *Cancer Cell Int*.; 16: 82 (2016).

Cotterill S.J., Parker L., More L., Craft A.W. Neuroblastoma: Changing incidence and survival in young people aged 0–24 years. A report from the north of England young persons' malignant disease registry. *Med. Pediatr. Oncol*. ;36:231–234 (2001).

Crisp M., Liu Q., Roux K., Rattner J.B., Shanahan C., Burke B., Stahl P.D., Hodzic D. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol.* Jan 2; 172(1):41-53 (2006).

Davidson P.M., Lammerding J. Broken nuclei – lamins, nuclear mechanics, and deseases. *Cell Press, XX* 1-10 (2013).

De Las Heras J.I. *et al*. Cancer biology and the nuclear envelope: a convoluted relationship. *Semin. Cancer Biol*. 23, 125–137 (2012).

Debes JD, Sebo TJ, Heemers HV, Kipp BR, Haugen DL, Lohse CM, Tindall DJ p300 modulates nuclear morphology in prostate cancer. *Cancer Res.* Feb 1; 65(3):708-12 (2005).

Dechat T., Korbei B., Vaughan O.A., Vlcek S., Hutchison C.J., *et al.* Lamina-associated polypeptide 2alpha binds intranuclear A-type lamins. *J Cell Sci* 113: 3473–3484 (2000).

Delanote V., Vandekerckhove J., Gettemans J., Plastins: versatilemodulators of actin organization in (patho)physiological cellular processes. *Acta Pharmacol Sin* 26: 769–779 (2005).

Diskin S.J., Hou C., Glessner J.T., et al. Copy number variation at 1q21. 1 associated with neuroblastoma. *Nature.* 459:987–91 (2009).

Dittmer T.A., Sahni N., Kubben N. Hill D. E., Vidal M., Burgess R.C., Roukos V., Mistelia T., Systematic identification of pathological lamin A interactors. *Mol Biol Cell.* May 1; 25(9): 1493–1510 (2014).

Dong S.W., Zhang H., Wang B.L., Sun P., Wang Y.G., Zhang P. Effect of the downregulation of SMYD3 expression by RNAi on RIZ1 expression and proliferation of esophageal squamous cell carcinoma. *Oncol Rep*. Sep; 32(3):1064-70 (2014).

Eriksson M. *et al.* Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423, 293–298 (2003).

Etchevers H. C., Amiel J., Lyonnet S., Molecular bases of human neurocrestopathies. *Adv Exp Med Biol* 589: 213-234 (2006).

Fisher D.Z., Chaudhary N., and Blobel G. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. Natl. Acad. Sci.* 83: 6450–6454 (1986).

Foran E., McWilliam P., Kelleher D., Croke D.T., Long A. The leukocyteprotein L-plastin induces proliferation, invasion and loss of E-cadherinexpression in colon cancer cells. *Int J Cancer* 118: 2098–2104 (2006).

Foster C.R., Przyborski S.A., Wilson R.G., et al. Lamins as cancer biomarkers [J] *Biochem Soc Trans*.;38(Pt 1):297–300 (2010).

Friedman R. C., et al., Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19(1): 92-105 (2009).

Furukawa K., Inagaki H., and Hotta Y. Identification and cloning of an mRNA coding for a germ cell-specific A- type lamin in mice. *Exp. Cell Res.* 212, 426–430 (1994).

Geisler, S.; Coller, J. RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. *Nat. Rev. Mol. Cell Biol.* 14, 699–712 (2013).

Gerace L., Comeau C. and Benson M. Organization and modulation of nuclear lamina structure. *J. Cell. Sci. Suppl. 1*, 137-160 (1984).

Gonzalez J.M., Pla D., Perez-Sala D., Andres V. A-type lamins and Hutchinson-Gilford progeria syndrome: pathogenesis and therapy. Front Biosci (Schol Ed). Jun 1; 3():1133-46 (2011).

Greene N. D. and Copp A. J., Development of the vertebrate central nervous system: formation of the neural tube. *Prenat Diagn* 29(4): 303-311 (2009).

Gruenbaum Y., Margalit A., Goldman R.D., Shumaker D.K., & Wilson K.L. The nuclear lamina comes of age. *Nat. Rev. Mol. Cell. Biol.* 6, 21-31 (2005).

Guarnieri D. J. and DiLeone R. J., MicroRNAs: a new class of gene regulators. *Ann Med* 40(3): 197-208. (2008).

Hamid Q.A., Fatima S., Thanumalayan S., Parnaik V.K., Activation of the lamin A gene during rat liver development, *FEBS Lett*. 392137–142 (1996).

Herrmann H., Aebi U. Intermediate filaments: molecular structure, assembly mechanism, and integration into functionally distinct intracellular scaffolds. *AnnuRev Biochem* 73, 749–789 (2004).

Herrmann H., Strelkov S.V. History and phylogeny of intermediate filaments: now in insects. *BMC Biol* 28, 16 (2011).

Hisama F.M., Lessel D., Leistritz D. et al. Coronary artery disease in a Werner syndrome-like form of progeria characterized by low levels of progerin, a splice variant of lamin A. *Am J Med Genet* A;155A: 3002–3006 (2011).

Hoeller D., Dikic I. Targeting the ubiquitin system in cancer therapy. *Nature*. Mar 26; 458(7237):438-44 (2009).

Holtz D., Tanaka R. A., Hartwig J. and McKeon F. The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. *Cell* 59, 969-977 (1989).

Horton H., McMorrow I. and Burke B. Independent expression and assembly properties of heterologous lamins A and C in murine embryonal carcinomas. *Eur. J. Cell Biol.* 57, 172-183 (1992).

Houben F., Ramaekers F.C., Snoeckx L.H., and Broers J.L. Role of nuclear lamina-cytoskeleton interactions in the maintenance of cellular strength. *Biochim. Biophys. Acta*1773, 675-686 (2007).

Huang Y., et al., Biological functions of microRNAs: a review. *J Physiol Biochem* 67(1): 129-139. (2011).

Hutchison C.J. Lamins: building blocks or regulators of gene expression? Nat. Rev. *Mol. Cell Biol*. 3, 848-858 (2002).

Janaki Ramaiah M., Parnaik V.K. *Biochemical and Biophysical Research Communications* 348 1132–1137 (2006).

Jansen M.P., Reijm E.A., Sieuwerts A.M., Ruigrok-Ritstier K., Look M.P., Rodríguez-González F.G., Heine A.A., Martens J.W., Sleijfer S., Foekens J.A., Berns E.M. High miR-26a and low CDC2 levels associate with decreased EZH2 expression and with favorable outcome on tamoxifen in metastatic breast cancer. *Breast Cancer Res Treat*. Jun; 133(3):937-47 (2012).

Jansson M.D., Lund A.H. MicroRNA and cancer. *Mol Oncol.* Dec; 6(6):590-610 (2012).

Ketema M., Wilhelmsen K., Kuikman I., Janssen H., Hodzic D., and Sonnenberg A. Requirements for the localization of nesprin-3 at the nuclear envelope and its interaction with plectin. *J. Cell Sci.*120, 3384-3394 (2007).

Ketema M., Kreft M., Secades P., Janssen H., Sonnenberg A. Nesprin-3 connects plectin and vimentin to the nuclear envelope of Sertoli cells but is not required for Sertoli cell function in spermatogenesis. *Mol Biol Cell.* Aug; 24(15):2454-66 (2013).

Kim V. N., Han J. and Siomi M. C., Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 10(2): 126-139 (2009).

Kitten G. T. and Nigg E. A. The CaaX motif is required for isoprenylation, carboxyl methylation, and nuclear-membrane association of lamin B2. *J. Cell Biol.* 113, 13-23 (1991).

Knudson A.G., Jr, Strong L.C. Mutation and cancer: neuroblastoma and pheochromocytoma. *Am J Hum Genet*. ;24:514–32 (1972).

Kong L. *et al.* Lamin A/C protein is overexpressed in tissue-invading prostate cancer and promotes prostate cancer cell growth, migration and invasion through the PI3K/AKT/PTEN pathway. *Carcinogenesis* 33, 751–759 (2012).

Kozomara A. and Griffiths-Jones S., miRBase: integrating microRNA annotation and deepsequencing data. *Nucleic Acids Res* 39: D152-D157 (2011).

LaBonne C. and Bronner-Fraser M., Molecular mechanisms of neural crest formation. *Annu Rev Cell Dev Biol* 15: 81-112. (1999).

Lammerding J., Schulze P.C., Takahashi T., Kozlov S., Sullivan T., et al. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction.*J Clin Invest* 113: 370–378 (2004).

Lewis B. P., Burge C. B. and Bartel D. P., Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120(1): 15-20 (2005).

Lin F., Worman H.J. Expression of nuclear lamins in human tissues and cancer cell lines and transcription from the promoters of the lamin A/C and B1 genes [J] *Exp Cell Res*. ;236(2):378–384 (1997).

Lin F., Morrison J.M., Wu W., Worman H.J., MAN1, an integral protein ofthe inner nuclear membrane, binds Smad2 and Smad3 and antagonizestransforming growth factor-beta signaling. *Hum Mol Genet* 14: 437–445 (2005).

Liu J., Ben-Shahar T.R., Riemer D., Treinin M., Spann P., Weber K., Fire A., and Gruenbaum Y. Essential roles for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. *Mol. Biol. Cell* 11: 3937–3947 (2000).

López-Otín C., Blasco M.A., Partridge L., Serrano M., Kroemer G. The hallmarks of aging. *Cell*. Jun 6; 153(6):1194-217 (2013).

Lussi Y.C., Hugi I., Laurell E., Kutay U. and Fahrenkrog B. Thenucleoporin Nup88 is interacting with nuclear lamin A. *Mol. Biol. Cell*,22, 1080–1090 (2011).

Ly D.H., Lockhart D.J., Lerner R.A., Schultz P.G., Mitotic misregulation and human aging. *Science* 287, 2486–2492 (2000).

Machiels B.M., Zorenc A.H., Endert J.M., Kuijpers H.J., van Eys G.J., Ramaekers F.C., and Broers J.L. An alternative splicing product of the lamin A/C gene lacks exon 10. *J. Biol. Chem.* 271: 9249–9253 (1996).

Machiels B.M., Broers J.L., Raymond Y., de Ley L., Kuijpers H.J., Caberg N.E., Ramaekers F.C. Eur J. Abnormal A-type lamin organization in a human lung carcinoma cell line. *Cell Biol.* Aug; 67(4):328-35 (1995).

Mall M., Walter T., Gorjánácz M., Davidson I.F., Nga Ly-Hartig T.B., Ellenberg J., Mattaj I.W. Mitotic lamin disassembly is

triggered by lipid-mediated signaling. *J Cell Biol*. Sep 17; 198(6):981-90 (2012).

Maresca G. *et al*. LMNA knock-down affects differentiation and progression of human neuroblastoma cells. *PLoSOne*, 7(9):e45513 (2012).

Maris J. M., Recent advances in neuroblastoma. *N Engl J Med* 362(23): 2202-2211. (2010).

Maris J.M., Mosse Y.P., Bradfield J.P., et al. Chromosome 6p22 locus associated with clinically aggressive neuroblastoma. *N Engl J Med.*; 358:2585–93 (2008).

Markiewicz E., Dechat T., Foisner R., Quinlan R.A., Hutchison CJ LaminA/C binding protein LAP2alpha is required for nuclear anchorage ofretinoblastoma protein. *Mol Biol Cell* 13: 4401–4413 (2002).

Markiewicz E., Tilgner K., Barker N., van de Wetering M., Clevers H., et al. The inner nuclear membrane protein emerin regulates beta-catenin activity byrestricting its accumulation in the nucleus. *Embo J* 25: 3275–3285 (2006).

Méjat A, Misteli T. LINC complexes in health and disease. *Nucleus.* Jan-Feb; 1(1):40-52 (2010)

Modak S., Cheung N. V., Neuroblastoma: Therapeutic strategies for a clinical enigma. *Cancer Treat Rev* 36(4): 307-317 (2010).

Moir R. D. and Goldman R. D. Lamin dynamics. *Curr. Opin. Cell Biol.* 5, 408-411 (1993).

Moir R.D., Spann T.P., Herrmann H., and Goldman R.D. Disruption of nuclear lamin organization blocks the elongation phase of DNAreplication. *J. Cell Biol.*149, 1179-1192 (2000).

Mosse Y.P., Laudenslager M., Khazi D., et al. Germline PHOX2B mutation in hereditary neuroblastoma. *Am J Hum Genet*. ;75:727–30. (2004)

Mossé Y.P., Laudenslager M., Longo L., et al. Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature.* ;455:930–5. (2008)

Mueller S. and Matthay K. K., Neuroblastoma: biology and staging. *Curr Oncol Rep* 11(6): 431-438. (2009).

Muralikrishna B. and Veena K. Parnaik. SP3 and AP-1 mediate transcriptional activation of the lamin Aproximal promoter.*Eur. J. Biochem*268, 1-9 (2001).

Nakajima N., Abe K., Genomic structure of the mouse A-type lamingene locus encoding somatic and germ cell-specific lamins, *FEBS Lett*.365 108–114 (1995).

Naeem A.S., Zhu Y., Di W.L., Marmiroli S., OShaughnessy R.F. AKT1-mediated Lamin A/C degradation is required for nuclear degradation and normal epidermal terminal differentiation. *Cell Death Differ* 22:2123-2132 (2015).

Namrata Rastogi, Shivali Duggal, Shailendra Kumar Singh, Konica Porwal, Vikas Kumar Srivastava, Rakesh Maurya, Madan L.B. Bhatt, and Durga Prasad Mishra Proteasome inhibition mediates p53 reactivation and anti-cancer activity of 6-Gingerol in cervical cancer cells *Oncotarget*. Dec 22; 6(41): 43310–43325 (2015).

Okumura K., Nakamachi K., Hosoe Y. and Nakajima N. Identification of a novel retinoic acid-responsive element withinthe lamin A/C promoter. *Biochem. Biophys. Res. Commun*. 269,197-202. (2000).

Orlowski R.Z., Kuhn D.J. Proteasome inhibitors in cancer therapy: lessons from the first decade. *Clin Cancer Res*. Mar 15; 14(6):1649-57 (2008).

Osmanagic-Myers S., Dechat T., Foisner R. Lamins at the crossroads of mechanosignaling. *Genes Dev*. Feb 1;29(3):225-37 (2015).

Padmakumar V.C., Abraham S., Braune S., Noegel A.A., Tunggal B., et al. Enaptin, a giant actin-binding protein, is an element of the nuclear membraneand the actin cytoskeleton. *Exp Cell Res* 295: 330–339 (2004).

Park N. J., et al., microRNA: discovery, characterization, and clinical utility for oral cancer detection. *Clin Cancer Res* 15(17): 5473-5477 (2009).

Peifer M., Hertwig F., Roels F., Dreidax D., Gartlgruber M., Menon R. et al. Telomerase activation by genomic rearrangements in high-risk neuroblastoma. *Nature*; 526: 700–704 (2015).

Peric-Hupkes D. *et al*. Molecular maps of the reorganization of genome–nuclear lamina interactions during differentiation. *Mol. Cell* 38, 603–613 (2010).

Peserico A., Germani A., Sanese P., Barbosa A.J., di Virgilio V., Fittipaldi R., Fabini E., Bertucci C., Varchi G., Moyer M.P., Caretti G., del Rio A., Simone C. J Cell Physiol. A SMYD3 Small-Molecule Inhibitor Impairing Cancer Cell Growth. Oct; 230(10):2447-60 (2015).

Pollard K.M., Chan E.K., Grant B.J., Sullivan K.F., Tan E.M., and Glass C.A. In vitro posttranslational modification of lamin B cloned from a human T-cell line. *Mol. Cell. Biol.*10: 2164–2175 (1990).

Prokocimer M., Davidovich M., Nissim-Rafinia M., Wiesel-Motiuk N., Bar D.Z., Barkan R., Meshorer E., and Gruenbaum Y. Nuclear lamins: key regulators of nuclear structure and activities. *J. Cell Mol. Med*. *13*, 1059-1085 (2009).

Reddy K.L., Zullo J.M., Bertolino E., and Singh H. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. *Nature 452*, 243-247 (2008).

Sakthivel K.M., Sehgal P. A Novel Role of Lamins from Genetic Disease to Cancer Biomarkers. *Oncol Rev*. Nov 14;10(2):309 (2016).

Sankpal U.T., Goodison S., Abdelrahim M., Basha R. Targeting Sp1 transcription factors in prostate cancer therapy. *Med Chem*. Sep; 7(5):518-25 (2011).

Sasseville A.M., Raymond Y., Lamin A precursor is localizated to intranuclear foci. *J. of Cell Science*. 108, 273-285 (1995).

Schirmer E.C. and Foisner R., Proteins that associate with lamins: many faces, many functions. *Exp. Cell Res.*313, 2167-2179 (2007).

Schultz D.C., Ayyanathan K., Negorev D., Maul G.G., Rauscher F.J. 3rd SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev*. Apr 15; 16(8):919-32 (2002).

Simon D.N. and Wilson K.L., Partners and post-translational modifications of nuclear lamins. *Chromosoma* 122, 13–31 (2013).

Siomi H. and Siomi M. C., On the road to reading the RNA-interference code. *Nature* 457(7228): 396-404 (2009).

Smythe C., Jenkins H.E., Hutchison C.J., Incorporation of the nuclear pore basket protein nup153 into nuclear pore structures is

dependent upon lamina assembly: evidence from cell-free extracts of Xenopus eggs. *EMBO J* 19, 3918–3931 (2000).

Spann T.P., Goldman A.E., Wang C., Huang S., and Goldman R.D., Alteration of nuclear lamin organization inhibits RNA polymerase II-dependent transcription. *J. Cell Biol. 156*, 603-608 (2002).

Spix C., Pastore G., Sankila R., Stiller C.A., Steliarova-Foucher E. Neuroblastoma incidence and survival in European children. Report from the automated childhood cancer information system project.Eur. J. *Cancer*. 2006;42:2081–2091 (1978–1997).

Subarna Dutta, Maitree Bhattacharyya, and Kaushik Sengupta. Implications and Assessment of the Elastic Behavior of Lamins in Laminopathies. *Cells*. 2016 Dec; 5(4): 37 (2016).

Suske G., The Sp-family of transcription factors. *Gene* 238,291-300 (1999).

Takahashi Y., et al., Orexin neurons are indispensable for prostaglandin E2-induced fever and defence against environmental cooling in mice. *J Physiol* 591(22): 5623-5643 (2013).

Tiwari B., MuralikrishnaBh., ParnaikV.K., Functional analysis ofthe 50 promoter region of the rat lamin A gene. *DNA Cell Biol*. 17957–965 (1998).

Trochet D., Bourdeaut F., Janoueix-Lerosey I., et al. Germ-line mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. *Am J Hum Genet*. 74:761–4 (2004).

Van Berlo J.H., Voncken J.W., Kubben N., Broers J.L., Duisters R., et al. Atypelamins are essential for TGF-beta1 induced PP2A to dephosphorylatetranscription factors. *Hum Mol Genet* 14: 2839–2849 (2005).

Vidak S. and Foisner R. Molecular insights into the premature aging disease progeria. *Histochem Cell Biol*.; 145: 401–417 (2016).

Wagner N., Krohne G., LEM-Domain proteins: new insights into lamininteractingproteins. *Int Rev Cytol* 261: 1–46 (2007).

Weber J. A., et al., The microRNA spectrum in 12 body fluids. *Clin Chem* 56(11): 1733-1741. (2010).

Willis N.D., Cox T.R., Rahman-Casañs S.F., Smits K., Przyborski S.A., van den Brandt P., van Engeland M., Weijenberg M., Wilson R.G., de Bruïne A., Hutchison C.J. Lamin A/C is a risk biomarker in colorectal cancer. *PLoS One*. Aug 20; 3(8):e2988 (2008).

Worman H.J., Schirmer E.C. Nuclear membrane diversity: underlying tissue-specific pathologies in disease? *Curr Opin Cell Biol*. Jun;34:101-12 (2015).

Xing Guo, Xiaorong Wang, Zhiping Wang, Sourav Banerjee, Jing Yang, Lan Huang, and Jack E. Dixon Site-specific Proteasome Phosphorylation Controls Cell Proliferation and Tumorigenesis *Nat Cell Biol.* Feb; 18(2): 202–212 (2016).

Zhu S., Thomas Look A. Neuroblastoma and Its Zebrafish Model. *Adv Exp Med Biol*.; 916:451-78 (2016).

# LIST OF PUBLICATIONS:

1. **Confocal Analysis of Nuclear Lamina Behavior During Male Meiosis and Spermatogenesis in *Drosophila melanogaster.*** Fabbretti F, Iannetti I, Guglielmi L, Perconti S, Evangelistella C, Proietti De Santis L and Prantera G. *PlosONE*, 2016 Mar 10;11(3):e0151231. doi: 10.1371/journal.pone.0151231.

2. **Lamin A/C Is Required For ChAT-Dependent Neuroblastoma Differentiation.** Guglielmi L, Nardella M, Musa C, Iannetti I, Arisi I, D'Onofrio M, Storti A, Valentini A, Cacci A, Biagioni S, Augusti-Tocco G, D'Agnano I, Felsani A. *MolecularNeurobiology*, 2016 May 25. doi:10.1007/s12035-016-9902-6.

3. **Down-regulation of the Lamin A/C in neuroblastoma triggers the expansion of tumor initiating cells.** Nardella M, Guglielmi L, Musa C, Iannetti I, Maresca G, Amendola

D, Porru M, Carico E, Sessa G, Camerlingo R, Dominici C, Megiorni F, Milan M, Bearzi C, Rizzi R, Pirozzi G, Leonetti C, Bucci B, Mercanti D, Felsani A and D'Agnano I. *Oncotarget*, 2015, Oct 20; 6(32):32821-40. doi: 10.18632/oncotarget.5104.

# APPENDIX 1

**TaqMan Human microRNA Array A v. 3.0 (Applied Biosystems).**

| | | LAN-5 | | SH-SY5Y | |
|---|---|---|---|---|---|
| **Pos** | **Detector** | **Ct** | **ΔCt** | **Ct** | **ΔCt** |
| A1 | hsa-let-7a-4373169 | 23,10 | 6,36 | 18,70 | 2,12 |
| A2 | hsa-let-7c-4373167 | 28,06 | 11,32 | 20,93 | 4,36 |
| A3 | hsa-let-7d-4395394 | 24,49 | 7,75 | 19,89 | 3,32 |
| A4 | hsa-let-7e-4395517 | 20,91 | 4,17 | 16,96 | 0,39 |
| A5 | hsa-let-7f-4373164 | 27,61 | 10,87 | 23,85 | 7,28 |
| A6 | hsa-let-7g-4395393 | 22,41 | 5,67 | 18,96 | 2,39 |
| A7 | hsa-miR-1-4395333 | 30,14 | 13,40 | 31,71 | 15,14 |
| A8 | hsa-miR-9-4373285 | 19,18 | 2,44 | 18,12 | 1,55 |
| A9 | hsa-miR-10a-4373153 | 29,35 | 12,61 | 26,02 | 9,44 |
| A10 | hsa-miR-10b-4395329 | 21,56 | 4,82 | 18,53 | 1,96 |
| A11 | MammU6-4395470 | 17,08 | | 17,11 | |
| A12 | MammU6-4395470 | 17,34 | | 17,11 | |
| A13 | hsa-miR-15a-4373123 | 23,32 | 6,58 | 23,54 | 6,97 |
| A14 | hsa-miR-15b-4373122 | 19,63 | 2,89 | 18,16 | 1,59 |
| A15 | hsa-miR-16-4373121 | 17,39 | 0,65 | 16,90 | 0,33 |
| A16 | hsa-miR-17-4395419 | 14,20 | -2,54 | 14,23 | -2,34 |
| A17 | hsa-miR-18a-4395533 | 19,32 | 2,58 | 19,77 | 3,20 |
| A18 | hsa-miR-18b-4395328 | 24,86 | 8,12 | 25,24 | 8,67 |
| A19 | hsa-miR-19a-4373099 | 19,11 | 2,37 | 20,38 | 3,81 |
| A20 | hsa-miR-19b-4373098 | 15,39 | -1,35 | 15,68 | -0,90 |

| | | | | | |
|---|---|---|---|---|---|
| A21 | hsa-miR-20a-4373286 | 15,46 | -1,28 | 15,23 | -1,34 |
| A22 | hsa-miR-20b-4373263 | 17,59 | 0,85 | 17,19 | 0,62 |
| A23 | hsa-miR-21-4373090 | 24,35 | 7,61 | 20,27 | 3,70 |
| A24 | hsa-miR-22-4373079 | 29,24 | 12,50 | 26,61 | 10,04 |
| B1 | hsa-miR-23a-4373074 | Undetermined | | 34,02 | 17,44 |
| B2 | hsa-miR-23b-4373073 | 23,86 | 7,12 | 24,22 | 7,65 |
| B3 | hsa-miR-24-4373072 | 15,78 | -0,96 | 15,10 | -1,47 |
| B4 | hsa-miR-25-4373071 | 19,10 | 2,36 | 17,66 | 1,09 |
| B5 | hsa-miR-26a-4395166 | 20,28 | 3,54 | 18,49 | 1,92 |
| B6 | hsa-miR-26b-4395167 | 23,09 | 6,35 | 20,85 | 4,28 |
| B7 | hsa-miR-27a-4373287 | 23,05 | 6,31 | 20,49 | 3,92 |
| B8 | hsa-miR-27b-4373068 | 21,21 | 4,47 | 19,75 | 3,18 |
| B9 | hsa-miR-28-3p-4395557 | 21,74 | 5,00 | 23,04 | 6,47 |
| B10 | hsa-miR-28-5p-4373067 | 24,48 | 7,74 | 23,82 | 7,25 |
| B11 | MammU6-4395470 | 17,38 | | 17,24 | |
| B12 | MammU6-4395470 | 16,84 | | 16,74 | |
| B13 | hsa-miR-29a-4395223 | 22,68 | 5,94 | 21,42 | 4,85 |
| B14 | hsa-miR-29b-4373288 | 30,77 | 14,03 | 28,27 | 11,69 |
| B15 | hsa-miR-29c-4395171 | 27,63 | 10,89 | 25,59 | 9,02 |
| B16 | hsa-miR-30b-4373290 | 18,59 | 1,85 | 18,03 | 1,46 |
| B17 | hsa-miR-30c-4373060 | 17,80 | 1,06 | 17,21 | 0,64 |
| B18 | hsa-miR-31-4395390 | Undetermined | | Undetermined | |
| B19 | hsa-miR-32-4395220 | 28,88 | 12,14 | 29,38 | 12,81 |
| B20 | hsa-miR-33b-4395196 | Undetermined | | Undetermined | |
| B21 | hsa-miR-34a-4395168 | 25,55 | 8,81 | 18,87 | 2,29 |
| B22 | hsa-miR-34c-5p-4373036 | 32,25 | 15,51 | 26,86 | 10,29 |
| B23 | hsa-miR-92a-4395169 | 16,22 | -0,52 | 16,51 | -0,06 |
| B24 | hsa-miR-93-4373302 | 17,00 | 0,26 | 15,51 | -1,07 |
| C1 | hsa-miR-95-4373011 | 24,35 | 7,61 | 22,42 | 5,85 |
| C2 | hsa-miR-96-4373372 | 30,07 | 13,33 | Undetermined | |
| C3 | hsa-miR-98-4373009 | 30,31 | 13,57 | 25,87 | 9,30 |

| | | | | | |
|---|---|---|---|---|---|
| C4 | hsa-miR-99a-4373008 | 27,64 | 10,90 | 21,96 | 5,38 |
| C5 | hsa-miR-99b-4373007 | 20,73 | 3,99 | 20,03 | 3,46 |
| C6 | hsa-miR-100-4373160 | 26,83 | 10,09 | 21,32 | 4,75 |
| C7 | hsa-miR-101-4395364 | 26,13 | 9,39 | 24,61 | 8,04 |
| C8 | hsa-miR-103-4373158 | 20,29 | 3,55 | 19,68 | 3,10 |
| C9 | hsa-miR-105-4395278 | 26,86 | 10,12 | 27,04 | 10,47 |
| C10 | hsa-miR-106a-4395280 | 14,15 | -2,59 | 14,17 | -2,41 |
| C11 | RNU44-4373384 | 16,70 | | 16,34 | |
| C12 | hsa-miR-106b-4373155 | 18,86 | 2,12 | 17,02 | 0,45 |
| C13 | hsa-miR-107-4373154 | 25,04 | 8,30 | 24,06 | 7,48 |
| C14 | hsa-miR-122-4395356 | Undetermined | | 37,38 | 20,81 |
| C15 | hsa-miR-124-4373295 | 27,56 | 10,82 | 27,64 | 11,07 |
| C16 | hsa-miR-125a-3p-4395310 | 27,25 | 10,51 | 27,23 | 10,66 |
| C17 | hsa-miR-125a-5p-4395309 | 17,63 | 0,89 | 17,89 | 1,31 |
| C18 | hsa-miR-125b-4373148 | 21,35 | 4,61 | 19,13 | 2,56 |
| C19 | hsa-miR-126-4395339 | 30,15 | 13,41 | 18,92 | 2,35 |
| C20 | hsa-miR-127-3p-4373147 | 17,15 | 0,41 | 29,42 | 12,85 |
| C21 | hsa-miR-127-5p-4395340 | 31,64 | 14,90 | Undetermined | |
| C22 | hsa-miR-128-4395327 | 23,45 | 6,71 | 22,90 | 6,33 |
| C23 | hsa-miR-129-3p-4373297 | 28,98 | 12,24 | 26,90 | 10,33 |
| C24 | hsa-miR-129-5p-4373171 | 34,02 | 17,28 | 28,60 | 12,03 |
| D1 | hsa-miR-130a-4373145 | 21,17 | 4,43 | 20,23 | 3,66 |
| D2 | hsa-miR-130b-4373144 | 20,03 | 3,29 | 20,17 | 3,60 |
| D3 | hsa-miR-132-4373143 | 18,96 | 2,22 | 18,37 | 1,80 |
| D4 | hsa-miR-133a-4395357 | 21,60 | 4,86 | 25,29 | 8,72 |
| D5 | hsa-miR-133b-4395358 | 28,37 | 11,63 | 31,79 | 15,21 |
| D6 | hsa-miR-134-4373299 | 21,77 | 5,03 | 28,99 | 12,42 |
| D7 | hsa-miR-135a-4373140 | 24,99 | 8,24 | 23,89 | 7,32 |
| D8 | hsa-miR-135b-4395372 | 21,27 | 4,53 | 29,13 | 12,56 |
| D9 | hsa-miR-136-4373173 | 33,55 | 16,81 | Undetermined | |
| D10 | hsa-miR-137-4373301 | 24,30 | 7,56 | 19,88 | 3,31 |

| | | | | | |
|---|---|---|---|---|---|
| D11 | hsa-miR-138-4395395 | 21,26 | 4,52 | 17,36 | 0,79 |
| D12 | hsa-miR-139-3p-4395424 | 22,91 | 6,17 | 24,59 | 8,02 |
| D13 | hsa-miR-139-5p-4395400 | 17,57 | 0,83 | 19,33 | 2,76 |
| D14 | hsa-miR-140-3p-4395345 | 25,52 | 8,78 | 24,88 | 8,31 |
| D15 | hsa-miR-140-5p-4373374 | 21,33 | 4,59 | 20,15 | 3,58 |
| D16 | hsa-miR-141-4373137 | 33,57 | 16,83 | 34,98 | 18,40 |
| D17 | hsa-miR-142-3p-4373136 | 31,73 | 14,99 | 30,37 | 13,80 |
| D18 | hsa-miR-142-5p-4395359 | Undetermined | | Undetermined | |
| D19 | hsa-miR-143-4395360 | 24,78 | 8,04 | 21,33 | 4,76 |
| D20 | hsa-miR-145-4395389 | 20,66 | 3,92 | 18,74 | 2,16 |
| D21 | hsa-miR-146a-4373132 | 27,41 | 10,67 | 26,38 | 9,81 |
| D22 | hsa-miR-146b-3p-4395472 | 33,62 | 16,88 | 31,05 | 14,48 |
| D23 | hsa-miR-146b-5p-4373178 | 22,11 | 5,37 | 21,78 | 5,21 |
| D24 | hsa-miR-147b-4395373 | Undetermined | | Undetermined | |
| E1 | hsa-miR-148a-4373130 | 24,28 | 7,54 | 23,84 | 7,26 |
| E2 | hsa-miR-148b-4373129 | 25,82 | 9,08 | 25,82 | 9,25 |
| E3 | hsa-miR-149-4395366 | 16,81 | 0,07 | 17,29 | 0,72 |
| E4 | hsa-miR-150-4373127 | 28,03 | 11,29 | 27,40 | 10,83 |
| E5 | hsa-miR-152-4395170 | 22,83 | 6,09 | 19,36 | 2,79 |
| E6 | hsa-miR-153-4373305 | 37,36 | 20,62 | 32,62 | 16,05 |
| E7 | hsa-miR-154-4373270 | 31,13 | 14,39 | Undetermined | |
| E8 | hsa-miR-181a-4373117 | 23,90 | 7,16 | 22,31 | 5,73 |
| E9 | hsa-miR-181c-4373115 | 32,85 | 16,11 | 29,19 | 12,62 |
| E10 | hsa-miR-182-4395445 | 26,54 | 9,80 | 32,81 | 16,24 |
| E11 | RNU48-4373383 | 15,10 | | 14,90 | |
| E12 | hsa-miR-183-4395380 | 26,35 | 9,61 | 32,94 | 16,37 |
| E13 | hsa-miR-184-4373113 | 28,97 | 12,23 | 23,78 | 7,21 |
| E14 | hsa-miR-185-4395382 | 24,91 | 8,17 | 23,96 | 7,39 |
| E15 | hsa-miR-186-4395396 | 17,80 | 1,06 | 19,31 | 2,74 |
| E16 | hsa-miR-187-4373307 | 36,43 | 19,69 | 29,50 | 12,93 |
| E17 | hsa-miR-188-3p-4395217 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| E18 | hsa-miR-190-4373110 | 30,20 | 13,46 | 29,37 | 12,80 |
| E19 | hsa-miR-191-4395410 | 15,85 | -0,89 | 15,46 | -1,12 |
| E20 | hsa-miR-192-4373108 | 23,67 | 6,93 | 24,05 | 7,48 |
| E21 | hsa-miR-193a-3p-4395361 | 35,22 | 18,48 | 38,98 | 22,40 |
| E22 | hsa-miR-193a-5p-4395392 | 26,18 | 9,44 | 24,99 | 8,41 |
| E23 | hsa-miR-193b-4395478 | 17,91 | 1,17 | 19,74 | 3,16 |
| E24 | hsa-miR-194-4373106 | 24,40 | 7,66 | 24,18 | 7,61 |
| F1 | hsa-miR-195-4373105 | 24,24 | 7,50 | 20,17 | 3,59 |
| F2 | hsa-miR-196b-4395326 | Undetermined | | Undetermined | |
| F3 | hsa-miR-197-4373102 | 20,60 | 3,86 | 21,23 | 4,66 |
| F4 | hsa-miR-198-4395384 | Undetermined | | 36,48 | 19,91 |
| F5 | hsa-miR-199a-5p-4373272 | 34,31 | 17,57 | 25,65 | 9,08 |
| F6 | hsa-miR-199a-3p-4395415 | 25,28 | 8,54 | 17,70 | 1,13 |
| F7 | hsa-miR-199b-5p-4373100 | 39,46 | 22,72 | 26,76 | 10,19 |
| F8 | hsa-miR-200a-4378069 | Undetermined | | Undetermined | |
| F9 | hsa-miR-200b-4395362 | 38,42 | 21,68 | 35,59 | 19,02 |
| F10 | hsa-miR-200c-4395411 | 26,52 | 9,77 | 28,53 | 11,96 |
| F11 | hsa-miR-202-4395474 | Undetermined | | Undetermined | |
| F12 | hsa-miR-203-4373095 | 25,09 | 8,35 | 25,68 | 9,11 |
| F13 | hsa-miR-204-4373094 | 22,27 | 5,53 | 32,08 | 15,51 |
| F14 | hsa-miR-205-4373093 | 31,46 | 14,71 | 32,39 | 15,82 |
| F15 | hsa-miR-208b-4395401 | Undetermined | | Undetermined | |
| F16 | hsa-miR-210-4373089 | 21,28 | 4,54 | 20,85 | 4,28 |
| F17 | hsa-miR-214-4395417 | 27,15 | 10,41 | 17,28 | 0,70 |
| F18 | hsa-miR-215-4373084 | 26,24 | 9,50 | 34,75 | 18,18 |
| F19 | hsa-miR-216a-4395331 | Undetermined | | Undetermined | |
| F20 | hsa-miR-216b-4395437 | Undetermined | | Undetermined | |
| F21 | hsa-miR-217-4395448 | Undetermined | | Undetermined | |
| F22 | hsa-miR-218-4373081 | 16,38 | -0,36 | 15,59 | -0,98 |
| F23 | hsa-miR-219-5p-4373080 | Undetermined | | Undetermined | |
| F24 | hsa-miR-221-4373077 | Undetermined | | 24,97 | 8,40 |

| | | | | | |
|---|---|---|---|---|---|
| G1 | hsa-miR-222-4395387 | 29,48 | 12,74 | 21,79 | 5,21 |
| G2 | hsa-miR-223-4395406 | 30,71 | 13,97 | 29,55 | 12,98 |
| G3 | hsa-miR-224-4395210 | 26,89 | 10,15 | 31,62 | 15,05 |
| G4 | hsa-miR-296-3p-4395212 | Undetermined | | 28,29 | 11,72 |
| G5 | hsa-miR-296-5p-4373066 | 32,57 | 15,83 | 22,81 | 6,24 |
| G6 | hsa-miR-299-3p-4373189 | Undetermined | | Undetermined | |
| G7 | hsa-miR-299-5p-4373188 | 25,95 | 9,21 | Undetermined | |
| G8 | hsa-miR-301a-4373064 | 21,16 | 4,42 | 19,31 | 2,74 |
| G9 | hsa-miR-301b-4395503 | 22,30 | 5,56 | 21,68 | 5,11 |
| G10 | hsa-miR-302a-4378070 | 33,46 | 16,72 | 33,08 | 16,50 |
| G11 | ath-miR159a-4373390 | Undetermined | | Undetermined | |
| G12 | hsa-miR-302b-4378071 | 33,42 | 16,68 | 28,22 | 11,65 |
| G13 | hsa-miR-302c-4378072 | 33,96 | 17,22 | 33,06 | 16,49 |
| G14 | hsa-miR-320-4395388 | 17,85 | 1,11 | 17,26 | 0,69 |
| G15 | hsa-miR-323-3p-4395338 | 18,34 | 1,60 | 24,94 | 8,37 |
| G16 | hsa-miR-324-3p-4395272 | 20,64 | 3,90 | 21,16 | 4,59 |
| G17 | hsa-miR-324-5p-4373052 | 21,15 | 4,41 | 19,94 | 3,36 |
| G18 | hsa-miR-326-4373050 | 33,52 | 16,78 | 34,62 | 18,05 |
| G19 | hsa-miR-328-4373049 | 22,88 | 6,14 | 21,13 | 4,55 |
| G20 | hsa-miR-329-4373191 | 27,02 | 10,28 | 36,69 | 20,11 |
| G21 | hsa-miR-330-3p-4373047 | 27,11 | 10,37 | 25,87 | 9,30 |
| G22 | hsa-miR-330-5p-4395341 | 37,74 | 21,00 | 33,78 | 17,21 |
| G23 | hsa-miR-331-3p-4373046 | 18,21 | 1,47 | 18,26 | 1,69 |
| G24 | hsa-miR-331-5p-4395344 | 24,49 | 7,75 | 25,49 | 8,91 |
| H1 | hsa-miR-335-4373045 | 25,04 | 8,30 | 25,48 | 8,91 |
| H2 | hsa-miR-337-5p-4395267 | 25,19 | 8,45 | Undetermined | |
| H3 | hsa-miR-338-3p-4395363 | 31,73 | 14,99 | 27,58 | 11,01 |
| H4 | hsa-miR-339-3p-4395295 | 22,25 | 5,51 | 21,99 | 5,42 |
| H5 | hsa-miR-339-5p-4395368 | 22,38 | 5,64 | 22,89 | 6,32 |
| H6 | hsa-miR-340-4395369 | 22,95 | 6,21 | 23,12 | 6,55 |
| H7 | has-miR-155-4395459 | 23,49 | 6,75 | 26,50 | 9,93 |

| | | | | | |
|---|---|---|---|---|---|
| H8 | hsa-let-7b-4395446 | 28,54 | 11,80 | 19,59 | 3,02 |
| H9 | hsa-miR-342-3p-4395371 | 16,45 | -0,29 | 17,07 | 0,50 |
| H10 | hsa-miR-342-5p-4395258 | 35,00 | 18,26 | 32,34 | 15,77 |
| H11 | hsa-miR-345-4395297 | 17,57 | 0,83 | 18,22 | 1,65 |
| H12 | hsa-miR-361-5p-4373035 | 24,12 | 7,38 | 22,37 | 5,80 |
| H13 | hsa-miR-362-3p-4395228 | 28,54 | 11,80 | 28,32 | 11,75 |
| H14 | hsa-miR-362-5p-4378092 | 24,97 | 8,23 | 24,02 | 7,44 |
| H15 | hsa-miR-363-4378090 | 28,13 | 11,39 | 25,44 | 8,87 |
| H16 | hsa-miR-365-4373194 | 23,99 | 7,25 | 26,36 | 9,78 |
| H17 | hsa-miR-367-4373034 | 32,26 | 15,52 | 30,29 | 13,72 |
| H18 | hsa-miR-369-3p-4373032 | 30,19 | 13,45 | 36,67 | 20,10 |
| H19 | hsa-miR-369-5p-4373195 | 29,14 | 12,40 | 35,63 | 19,05 |
| H20 | hsa-miR-370-4395386 | 19,78 | 3,04 | 25,45 | 8,88 |
| H21 | hsa-miR-371-3p-4395235 | Undetermined | | Undetermined | |
| H22 | hsa-miR-372-4373029 | 29,50 | 12,76 | 32,92 | 16,34 |
| H23 | hsa-miR-373-4378073 | Undetermined | | Undetermined | |
| H24 | hsa-miR-374a-4373028 | 21,87 | 5,13 | 20,47 | 3,90 |
| I1 | hsa-miR-374b-4381045 | 20,38 | 3,64 | 19,47 | 2,90 |
| I2 | hsa-miR-375-4373027 | 17,85 | 1,11 | 18,26 | 1,69 |
| I3 | hsa-miR-376a-4373026 | 18,41 | 1,67 | 25,50 | 8,93 |
| I4 | hsa-miR-376b-4373196 | 31,16 | 14,42 | Undetermined | |
| I5 | hsa-miR-377-4373025 | Undetermined | | Undetermined | |
| I6 | hsa-miR-379-4373349 | 21,37 | 4,63 | 26,84 | 10,27 |
| I7 | hsa-miR-380-4373022 | 31,04 | 14,30 | Undetermined | |
| I8 | hsa-miR-381-4373020 | 29,73 | 12,99 | 36,04 | 19,46 |
| I9 | hsa-miR-382-4373019 | 20,41 | 3,67 | 25,12 | 8,55 |
| I10 | hsa-miR-383-4373018 | 26,08 | 9,34 | 29,79 | 13,22 |
| I11 | hsa-miR-409-5p-4395442 | 26,68 | 9,94 | 34,38 | 17,81 |
| I12 | hsa-miR-410-4378093 | 21,22 | 4,48 | 27,82 | 11,25 |
| I13 | hsa-miR-411-4381013 | 20,75 | 4,01 | 25,94 | 9,37 |
| I14 | hsa-miR-422a-4395408 | 29,76 | 13,02 | 32,42 | 15,84 |

| | | | | | |
|---|---|---|---|---|---|
| I15 | hsa-miR-423-5p-4395451 | 27,00 | 10,26 | 25,04 | 8,47 |
| I16 | hsa-miR-424-4373201 | 30,67 | 13,93 | 27,98 | 11,41 |
| I17 | hsa-miR-425-4380926 | 24,48 | 7,74 | 22,87 | 6,30 |
| I18 | hsa-miR-429-4373203 | Undetermined | | 39,15 | 22,58 |
| I19 | hsa-miR-431-4395173 | 19,54 | 2,80 | Undetermined | |
| I20 | hsa-miR-433-4373205 | 20,53 | 3,79 | 36,05 | 19,48 |
| I21 | hsa-miR-449a-4373207 | 36,64 | 19,90 | 29,94 | 13,37 |
| I22 | hsa-miR-449b-4381011 | Undetermined | | 31,14 | 14,57 |
| I23 | hsa-miR-450a-4395414 | 33,02 | 16,28 | 31,28 | 14,71 |
| I24 | hsa-miR-450b-3p-4395319 | Undetermined | | Undetermined | |
| J1 | hsa-miR-450b-5p-4395318 | 31,70 | 14,96 | 31,15 | 14,57 |
| J2 | hsa-miR-451-4373360 | Undetermined | | Undetermined | |
| J3 | hsa-miR-452-4395440 | 29,93 | 13,19 | Undetermined | |
| J4 | hsa-miR-453-4395429 | 27,12 | 10,38 | Undetermined | |
| J5 | hsa-miR-454-4395434 | 17,54 | 0,80 | 18,16 | 1,59 |
| J6 | hsa-miR-455-3p-4395355 | 27,70 | 10,96 | 24,77 | 8,19 |
| J7 | hsa-miR-455-5p-4378098 | 31,00 | 14,26 | 27,68 | 11,11 |
| J8 | hsa-miR-483-5p-4395449 | 27,27 | 10,53 | 22,95 | 6,38 |
| J9 | hsa-miR-484-4381032 | 15,37 | -1,37 | 16,25 | -0,33 |
| J10 | hsa-miR-485-3p-4378095 | 21,59 | 4,85 | 28,87 | 12,29 |
| J11 | hsa-miR-485-5p-4373212 | 29,10 | 12,36 | Undetermined | |
| J12 | hsa-miR-486-3p-4395204 | 38,59 | 21,85 | Undetermined | |
| J13 | hsa-miR-486-5p-4378096 | 26,05 | 9,31 | 27,94 | 11,37 |
| J14 | hsa-miR-487a-4378097 | 27,34 | 10,60 | 36,68 | 20,11 |
| J15 | hsa-miR-487b-4378102 | 22,32 | 5,58 | 28,90 | 12,32 |
| J16 | hsa-miR-488-4395468 | 27,56 | 10,82 | 29,06 | 12,49 |
| J17 | hsa-miR-489-4395469 | 26,57 | 9,83 | 24,48 | 7,91 |
| J18 | hsa-miR-490-3p-4373215 | 27,82 | 11,08 | 27,34 | 10,77 |
| J19 | hsa-miR-491-3p-4395471 | Undetermined | | Undetermined | |
| J20 | hsa-miR-491-5p-4381053 | 22,92 | 6,18 | 23,10 | 6,53 |
| J21 | hsa-miR-493-4395475 | 25,06 | 8,32 | 38,71 | 22,14 |

| | | | | | |
|---|---|---|---|---|---|
| J22 | hsa-miR-494-4395476 | 21,37 | 4,63 | 28,23 | 11,66 |
| J23 | hsa-miR-495-4381078 | 21,30 | 4,56 | 27,03 | 10,46 |
| J24 | hsa-miR-496-4386771 | 30,20 | 13,46 | Undetermined | |
| K1 | hsa-miR-499-3p-4395538 | Undetermined | | Undetermined | |
| K2 | hsa-miR-499-5p-4381047 | 36,70 | 19,96 | 37,37 | 20,80 |
| K3 | hsa-miR-500-4395539 | 24,93 | 8,19 | 24,27 | 7,69 |
| K4 | hsa-miR-501-3p-4395546 | 27,77 | 11,03 | 29,12 | 12,55 |
| K5 | hsa-miR-501-5p-4373226 | 24,58 | 7,84 | 24,23 | 7,66 |
| K6 | hsa-miR-502-3p-4395194 | 29,21 | 12,47 | 27,61 | 11,04 |
| K7 | hsa-miR-502-5p-4373227 | 29,44 | 12,69 | 28,07 | 11,50 |
| K8 | hsa-miR-503-4373228 | 30,43 | 13,69 | 27,91 | 11,33 |
| K9 | hsa-miR-504-4395195 | 24,70 | 7,96 | Undetermined | |
| K10 | hsa-miR-505-4395200 | 25,14 | 8,40 | 25,53 | 8,96 |
| K11 | hsa-miR-507-4373232 | Undetermined | | Undetermined | |
| K12 | hsa-miR-508-3p-4373233 | 32,34 | 15,60 | 39,03 | 22,46 |
| K13 | hsa-miR-508-5p-4395203 | Undetermined | | Undetermined | |
| K14 | hsa-miR-509-5p-4395346 | Undetermined | | 33,78 | 17,21 |
| K15 | hsa-miR-510-4395352 | Undetermined | | Undetermined | |
| K16 | hsa-miR-512-3p-4381034 | 32,11 | 15,37 | Undetermined | |
| K17 | hsa-miR-512-5p-4373238 | Undetermined | | Undetermined | |
| K18 | hsa-miR-513-5p-4395201 | Undetermined | | Undetermined | |
| K19 | hsa-miR-515-3p-4395480 | Undetermined | | Undetermined | |
| K20 | hsa-miR-515-5p-4373242 | Undetermined | | Undetermined | |
| K21 | hsa-miR-516a-5p-4395527 | Undetermined | | Undetermined | |
| K22 | hsa-miR-516b-4395172 | Undetermined | | Undetermined | |
| K23 | hsa-miR-517a-4395513 | 37,95 | 21,21 | Undetermined | |
| K24 | hsa-miR-517c-4373264 | Undetermined | | Undetermined | |
| L1 | hsa-miR-518a-3p-4395508 | Undetermined | | Undetermined | |
| L2 | hsa-miR-518a-5p-4395507 | Undetermined | | Undetermined | |
| L3 | hsa-miR-518b-4373246 | 35,31 | 18,57 | 31,95 | 15,37 |
| L4 | hsa-miR-518c-4395512 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| L5 | hsa-miR-518d-3p-4373248 | Undetermined | | Undetermined | |
| L6 | hsa-miR-518d-5p-4395500 | Undetermined | | Undetermined | |
| L7 | hsa-miR-518e-4395506 | Undetermined | | Undetermined | |
| L8 | hsa-miR-518f-4395499 | 25,26 | 8,52 | 24,59 | 8,02 |
| L9 | hsa-miR-519a-4395526 | Undetermined | | Undetermined | |
| L10 | hsa-miR-519d-4395514 | Undetermined | | Undetermined | |
| L11 | hsa-miR-519e-4395481 | Undetermined | | Undetermined | |
| L12 | hsa-miR-520a-3p-4373268 | Undetermined | | Undetermined | |
| L13 | hsa-miR-520a-5p-4378085 | Undetermined | | Undetermined | |
| L14 | hsa-miR-520d-5p-4395504 | Undetermined | | Undetermined | |
| L15 | hsa-miR-520g-4373257 | Undetermined | | Undetermined | |
| L16 | hsa-miR-521-4373259 | Undetermined | | Undetermined | |
| L17 | hsa-miR-522-4395524 | Undetermined | | Undetermined | |
| L18 | hsa-miR-523-4395497 | 27,24 | 10,50 | 28,94 | 12,37 |
| L19 | hsa-miR-524-5p-4395174 | Undetermined | | Undetermined | |
| L20 | hsa-miR-525-3p-4395496 | Undetermined | | Undetermined | |
| L21 | hsa-miR-525-5p-4378088 | Undetermined | | Undetermined | |
| L22 | hsa-miR-526b-4395493 | Undetermined | | Undetermined | |
| L23 | hsa-miR-532-3p-4395466 | 20,61 | 3,87 | 20,47 | 3,89 |
| L24 | hsa-miR-532-5p-4380928 | 20,26 | 3,52 | 19,72 | 3,15 |
| M1 | hsa-miR-539-4378103 | 18,57 | 1,83 | 24,32 | 7,75 |
| M2 | hsa-miR-541-4395312 | 26,02 | 9,28 | 39,21 | 22,64 |
| M3 | hsa-miR-542-3p-4378101 | 32,04 | 15,30 | 29,85 | 13,27 |
| M4 | hsa-miR-542-5p-4395351 | 30,29 | 13,55 | 31,17 | 14,60 |
| M5 | hsa-miR-544-4395376 | Undetermined | | Undetermined | |
| M6 | hsa-miR-545-4395378 | 31,77 | 15,03 | 29,16 | 12,59 |
| M7 | hsa-miR-548a-3p-4380948 | Undetermined | | Undetermined | |
| M8 | hsa-miR-548a-5p-4395523 | Undetermined | | Undetermined | |
| M9 | hsa-miR-548b-3p-4380951 | Undetermined | | Undetermined | |
| M10 | hsa-miR-548b-5p-4395519 | 30,30 | 13,56 | 31,12 | 14,55 |
| M11 | hsa-miR-548c-3p-4380993 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| M12 | hsa-miR-548c-5p-4395540 | 34,08 | 17,34 | 33,34 | 16,76 |
| M13 | hsa-miR-548d-3p-4381008 | 33,88 | 17,14 | 38,34 | 21,77 |
| M14 | hsa-miR-548d-5p-4395348 | 31,93 | 15,19 | 32,12 | 15,55 |
| M15 | hsa-miR-551b-4380945 | 31,24 | 14,50 | 28,27 | 11,69 |
| M16 | hsa-miR-556-3p-4395456 | Undetermined | | Undetermined | |
| M17 | hsa-miR-556-5p-4395455 | Undetermined | | Undetermined | |
| M18 | hsa-miR-561-4380938 | 37,01 | 20,27 | Undetermined | |
| M19 | hsa-miR-570-4395458 | 35,01 | 18,27 | 33,25 | 16,68 |
| M20 | hsa-miR-574-3p-4395460 | 23,15 | 6,41 | 19,95 | 3,38 |
| M21 | hsa-miR-576-3p-4395462 | 27,07 | 10,33 | 26,44 | 9,87 |
| M22 | hsa-miR-576-5p-4395461 | 36,77 | 20,03 | 33,85 | 17,27 |
| M23 | hsa-miR-579-4395509 | 28,70 | 11,96 | 29,83 | 13,26 |
| M24 | hsa-miR-582-3p-4395510 | Undetermined | | 33,62 | 17,05 |
| N1 | hsa-miR-582-5p-4395175 | Undetermined | | 33,97 | 17,40 |
| N2 | hsa-miR-589-4395520 | 31,56 | 14,82 | 31,47 | 14,90 |
| N3 | hsa-miR-590-5p-4395176 | 22,34 | 5,60 | 21,07 | 4,50 |
| N4 | hsa-miR-597-4380960 | 26,54 | 9,80 | 27,65 | 11,08 |
| N5 | hsa-miR-598-4395179 | 22,40 | 5,66 | 21,31 | 4,73 |
| N6 | hsa-miR-615-3p-4386777 | 27,01 | 10,27 | 26,46 | 9,89 |
| N7 | hsa-miR-615-5p-4395464 | 38,47 | 21,73 | 36,16 | 19,59 |
| N8 | hsa-miR-616-4395525 | 32,49 | 15,75 | 31,98 | 15,41 |
| N9 | hsa-miR-618-4380996 | 25,19 | 8,45 | 25,96 | 9,39 |
| N10 | hsa-miR-624-4395541 | Undetermined | | Undetermined | |
| N11 | hsa-miR-625-4395542 | 27,93 | 11,19 | 27,36 | 10,78 |
| N12 | hsa-miR-627-4380967 | 35,48 | 18,74 | 33,88 | 17,31 |
| N13 | hsa-miR-628-5p-4395544 | 23,20 | 6,46 | 23,44 | 6,87 |
| N14 | hsa-miR-629-4395547 | 26,50 | 9,76 | 28,23 | 11,65 |
| N15 | hsa-miR-636-4395199 | 25,55 | 8,81 | 26,32 | 9,74 |
| N16 | hsa-miR-642-4380995 | 25,32 | 8,58 | 26,53 | 9,96 |
| N17 | hsa-miR-651-4381007 | 36,58 | 19,84 | 36,11 | 19,54 |
| N18 | hsa-miR-652-4395463 | 21,31 | 4,57 | 21,67 | 5,10 |

| | | | | | |
|---|---|---|---|---|---|
| N19 | hsa-miR-653-4395403 | Undetermined | | Undetermined | |
| N20 | hsa-miR-654-3p-4395350 | 27,95 | 11,21 | Undetermined | |
| N21 | hsa-miR-654-5p-4381014 | 23,20 | 6,46 | 29,90 | 13,33 |
| N22 | hsa-miR-655-4381015 | 27,27 | 10,53 | 36,56 | 19,99 |
| N23 | hsa-miR-660-4380925 | 23,20 | 6,46 | 22,00 | 5,43 |
| N24 | hsa-miR-671-3p-4395433 | 23,71 | 6,97 | 23,58 | 7,01 |
| O1 | hsa-miR-672-4395438 | 33,54 | 16,80 | Undetermined | |
| O2 | hsa-miR-674-4395193 | Undetermined | | Undetermined | |
| O3 | hsa-miR-708-4395452 | 18,13 | 1,39 | 17,44 | 0,86 |
| O4 | hsa-miR-744-4395435 | 20,51 | 3,77 | 19,50 | 2,93 |
| O5 | hsa-miR-758-4395180 | 22,10 | 5,36 | 29,62 | 13,05 |
| O6 | hsa-miR-871-4395465 | Undetermined | | Undetermined | |
| O7 | hsa-miR-872-4395375 | Undetermined | | Undetermined | |
| O8 | hsa-miR-873-4395467 | 28,95 | 12,21 | 32,00 | 15,43 |
| O9 | hsa-miR-874-4395379 | 29,33 | 12,59 | 31,77 | 15,20 |
| O10 | hsa-miR-875-3p-4395315 | Undetermined | | Undetermined | |
| O11 | hsa-miR-876-3p-4395336 | 28,47 | 11,73 | 28,25 | 11,68 |
| O12 | hsa-miR-876-5p-4395316 | 29,80 | 13,06 | 30,66 | 14,08 |
| O13 | hsa-miR-885-3p-4395483 | Undetermined | | Undetermined | |
| O14 | hsa-miR-885-5p-4395407 | 22,69 | 5,95 | 22,49 | 5,92 |
| O15 | hsa-miR-886-3p-4395305 | Undetermined | | Undetermined | |
| O16 | hsa-miR-886-5p-4395304 | 33,17 | 16,43 | 37,94 | 21,37 |
| O17 | hsa-miR-887-4395485 | 34,44 | 17,70 | 31,72 | 15,15 |
| O18 | hsa-miR-888-4395323 | 33,85 | 17,11 | Undetermined | |
| O19 | hsa-miR-889-4395313 | 24,93 | 8,19 | 35,09 | 18,51 |
| O20 | hsa-miR-890-4395320 | Undetermined | | Undetermined | |
| O21 | hsa-miR-891a-4395302 | 35,79 | 19,05 | Undetermined | |
| O22 | hsa-miR-891b-4395321 | Undetermined | | Undetermined | |
| O23 | hsa-miR-892a-4395306 | Undetermined | | Undetermined | |
| O24 | hsa-miR-147-4373131 | 35,84 | 19,10 | 35,11 | 18,54 |
| P1 | hsa-miR-208-4373091 | 29,85 | 13,11 | 25,25 | 8,68 |

| | | | | | |
|---|---|---|---|---|---|
| P2 | hsa-miR-211-4373088 | Undetermined | | Undetermined | |
| P3 | hsa-miR-212-4373087 | 23,60 | 6,86 | 24,53 | 7,95 |
| P4 | hsa-miR-219-1-3p-4395206 | 30,96 | 14,22 | 29,81 | 13,24 |
| P5 | hsa-miR-219-2-3p-4395501 | Undetermined | | Undetermined | |
| P6 | hsa-miR-220-4373078 | Undetermined | | Undetermined | |
| P7 | hsa-miR-220b-4395317 | Undetermined | | Undetermined | |
| P8 | hsa-miR-220c-4395322 | Undetermined | | Undetermined | |
| P9 | hsa-miR-298-4395301 | Undetermined | | Undetermined | |
| P10 | hsa-miR-325-4373051 | Undetermined | | Undetermined | |
| P11 | hsa-miR-346-4373038 | 31,12 | 14,38 | 31,41 | 14,83 |
| P12 | hsa-miR-376c-4395233 | 20,14 | 3,40 | 26,75 | 10,17 |
| P13 | hsa-miR-384-4373017 | Undetermined | | Undetermined | |
| P14 | hsa-miR-412-4373199 | 34,49 | 17,75 | Undetermined | |
| P15 | hsa-miR-448-4373206 | 36,74 | 20,00 | Undetermined | |
| P16 | hsa-miR-492-4373217 | Undetermined | | Undetermined | |
| P17 | hsa-miR-506-4373231 | 33,77 | 17,03 | Undetermined | |
| P18 | hsa-miR-509-3-5p-4395266 | Undetermined | | Undetermined | |
| P19 | hsa-miR-511-4373236 | Undetermined | | Undetermined | |
| P20 | hsa-miR-517b-4373244 | Undetermined | | Undetermined | |
| P21 | hsa-miR-519c-3p-4373251 | Undetermined | | Undetermined | |
| P22 | hsa-miR-520b-4373252 | Undetermined | | 37,83 | 21,26 |
| P23 | hsa-miR-520e-4373255 | Undetermined | | Undetermined | |
| P24 | hsa-miR-520f-4373256 | 39,17 | 22,43 | 37,50 | 20,93 |

# APPENDIX 2

**TaqMan Human microRNA Array B v. 3.0 (Applied Biosystems).**

| | | LAN-5 | | SH-SY5Y | |
|---|---|---|---|---|---|
| **Pos** | **Detector** | **Ct** | **ΔCt** | **Ct** | **ΔCt** |
| A1 | dme-miR-7-000268 | 24,12 | 7,51 | 22,60 | 22,60 |
| A2 | hsa-miR-548I-002909 | Undetermined | | Undetermined | |
| A3 | hsa-miR-30a-3p-000416 | 20,81 | 4,21 | 20,32 | 20,32 |
| A4 | hsa-miR-30a-5p-000417 | 21,08 | 4,48 | 19,97 | 19,97 |
| A5 | hsa-miR-30d-000420 | 24,91 | 8,31 | 23,66 | 23,66 |
| A6 | hsa-miR-30e-3p-000422 | 20,88 | 4,28 | 19,89 | 19,89 |
| A7 | hsa-miR-34b-000427 | Undetermined | | 35,04 | 35,04 |
| A8 | hsa-miR-126#-000451 | Undetermined | | 25,32 | 25,32 |
| A9 | hsa-miR-154#-000478 | 25,78 | 9,17 | 32,04 | 32,04 |
| A10 | hsa-miR-182#-000483 | Undetermined | | Undetermined | |
| A11 | U6 snRNA-001973 | 17,28 | | | |
| A12 | U6 snRNA-001973 | 17,50 | | 17,42 | |
| A13 | hsa-miR-206-000510 | 32,07 | 15,46 | 29,35 | 29,35 |
| A14 | hsa-miR-213-000516 | 25,32 | 8,72 | 29,70 | 29,70 |
| A15 | hsa-miR-302c#-000534 | 31,90 | 15,29 | 36,27 | 36,27 |
| A16 | hsa-miR-302d-000535 | 32,05 | 15,45 | 32,90 | 32,90 |
| A17 | hsa-miR-378-000567 | 27,75 | 11,14 | 31,96 | 31,96 |
| A18 | hsa-miR-380-5p-000570 | 27,47 | 10,87 | 35,76 | 35,76 |
| A19 | hsa-miR-1257-002910 | 34,82 | 18,22 | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| A20 | hsa-miR-200a#-001011 | Undetermined | | Undetermined | |
| A21 | hsa-miR-432-001026 | 19,85 | 3,24 | 31,89 | 31,89 |
| A22 | hsa-miR-432#-001027 | 31,98 | 15,38 | Undetermined | |
| A23 | hsa-miR-497-001043 | Undetermined | | 25,19 | 25,19 |
| A24 | hsa-miR-500-001046 | 29,20 | 12,60 | 29,23 | 29,23 |
| B1 | hsa-miR-1238-002927 | Undetermined | | Undetermined | |
| B2 | hsa-miR-488-001106 | 29,84 | 13,24 | 30,63 | 30,63 |
| B3 | hsa-miR-517#-001113 | Undetermined | | Undetermined | |
| B4 | hsa-miR-516-3p-001149 | 34,73 | 18,13 | 36,54 | 36,54 |
| B5 | hsa-miR-518c#-001158 | Undetermined | | Undetermined | |
| B6 | hsa-miR-519e#-001166 | Undetermined | | Undetermined | |
| B7 | hsa-miR-520h-001170 | Undetermined | | Undetermined | |
| B8 | hsa-miR-524-001173 | Undetermined | | Undetermined | |
| B9 | mmu-let-7d#-001178 | Undetermined | | Undetermined | |
| B10 | hsa-miR-363#-001283 | 36,83 | 20,22 | 30,10 | 30,10 |
| B11 | U6 snRNA-001973 | 16,91 | | 16,91 | |
| B12 | U6 snRNA-001973 | 16,77 | | 18,63 | |
| B13 | rno-miR-7#-001338 | 20,29 | 3,69 | 20,86 | 20,86 |
| B14 | hsa-miR-656-001510 | 26,03 | 9,43 | Undetermined | |
| B15 | hsa-miR-549-001511 | Undetermined | | Undetermined | |
| B16 | hsa-miR-657-001512 | 36,76 | 20,16 | 37,54 | 37,54 |
| B17 | hsa-miR-658-001513 | Undetermined | | Undetermined | |
| B18 | hsa-miR-659-001514 | Undetermined | | 38,98 | 38,98 |
| B19 | hsa-miR-551a-001519 | Undetermined | | 36,51 | 36,51 |
| B20 | hsa-miR-552-001520 | Undetermined | | Undetermined | |
| B21 | hsa-miR-553-001521 | Undetermined | | Undetermined | |
| B22 | hsa-miR-554-001522 | Undetermined | | Undetermined | |
| B23 | hsa-miR-555-001523 | Undetermined | | Undetermined | |
| B24 | hsa-miR-557-001525 | Undetermined | | Undetermined | |
| C1 | hsa-miR-558-001526 | Undetermined | | Undetermined | |
| C2 | hsa-miR-559-001527 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| C3 | hsa-miR-562-001529 | Undetermined | | Undetermined | |
| C4 | hsa-miR-563-001530 | Undetermined | | Undetermined | |
| C5 | hsa-miR-564-001531 | 28,91 | 12,31 | 31,61 | 31,61 |
| C6 | hsa-miR-566-001533 | 38,53 | 21,93 | Undetermined | |
| C7 | hsa-miR-567-001534 | Undetermined | | Undetermined | |
| C8 | hsa-miR-569-001536 | Undetermined | | Undetermined | |
| C9 | hsa-miR-586-001539 | 33,46 | 16,86 | Undetermined | |
| C10 | hsa-miR-587-001540 | Undetermined | | Undetermined | |
| C11 | RNU44-001094 | 16,10 | | 16,20 | |
| C12 | hsa-miR-588-001542 | Undetermined | | Undetermined | |
| C13 | hsa-miR-589-001543 | 24,34 | 7,74 | 23,79 | 23,79 |
| C14 | hsa-miR-550-001544 | 30,13 | 13,53 | 34,11 | 34,11 |
| C15 | hsa-miR-591-001545 | Undetermined | | Undetermined | |
| C16 | hsa-miR-592-001546 | 20,07 | 3,47 | 23,89 | 23,89 |
| C17 | hsa-miR-593-001547 | Undetermined | | Undetermined | |
| C18 | hsa-miR-596-001550 | 34,36 | 17,76 | 33,56 | 33,56 |
| C19 | hsa-miR-622-001553 | Undetermined | | Undetermined | |
| C20 | hsa-miR-599-001554 | Undetermined | | Undetermined | |
| C21 | hsa-miR-623-001555 | Undetermined | | Undetermined | |
| C22 | hsa-miR-600-001556 | Undetermined | | Undetermined | |
| C23 | hsa-miR-624-001557 | 32,94 | 16,33 | 33,56 | 33,56 |
| C24 | hsa-miR-601-001558 | 31,45 | 14,85 | 29,87 | 29,87 |
| D1 | hsa-miR-626-001559 | Undetermined | | Undetermined | |
| D2 | hsa-miR-629-001562 | 24,27 | 7,66 | 25,80 | 25,80 |
| D3 | hsa-miR-630-001563 | 35,63 | 19,02 | Undetermined | |
| D4 | hsa-miR-631-001564 | Undetermined | | Undetermined | |
| D5 | hsa-miR-603-001566 | Undetermined | | Undetermined | |
| D6 | hsa-miR-604-001567 | 36,86 | 20,26 | 38,26 | 38,26 |
| D7 | hsa-miR-605-001568 | Undetermined | | Undetermined | |
| D8 | hsa-miR-606-001569 | Undetermined | | Undetermined | |
| D9 | hsa-miR-607-001570 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| D10 | hsa-miR-608-001571 | Undetermined | | Undetermined | |
| D11 | hsa-miR-609-001573 | Undetermined | | Undetermined | |
| D12 | hsa-miR-633-001574 | Undetermined | | Undetermined | |
| D13 | hsa-miR-634-001576 | Undetermined | | Undetermined | |
| D14 | hsa-miR-635-001578 | Undetermined | | Undetermined | |
| D15 | hsa-miR-637-001581 | Undetermined | | Undetermined | |
| D16 | hsa-miR-638-001582 | 32,21 | 15,61 | 31,18 | 31,18 |
| D17 | hsa-miR-639-001583 | 28,92 | 12,32 | 32,69 | 32,69 |
| D18 | hsa-miR-640-001584 | Undetermined | | Undetermined | |
| D19 | hsa-miR-641-001585 | 32,12 | 15,52 | 31,82 | 31,82 |
| D20 | hsa-miR-613-001586 | Undetermined | | Undetermined | |
| D21 | hsa-miR-614-001587 | 39,80 | 23,20 | Undetermined | |
| D22 | hsa-miR-616-001589 | 31,32 | 14,71 | 31,45 | 31,45 |
| D23 | hsa-miR-617-001591 | 36,79 | 20,18 | Undetermined | |
| D24 | hsa-miR-643-001594 | 36,36 | 19,75 | 31,66 | 31,66 |
| E1 | hsa-miR-644-001596 | Undetermined | | Undetermined | |
| E2 | hsa-miR-645-001597 | 36,20 | 19,60 | 32,84 | 32,84 |
| E3 | hsa-miR-621-001598 | Undetermined | | Undetermined | |
| E4 | hsa-miR-646-001599 | 28,00 | 11,40 | 27,25 | 27,25 |
| E5 | hsa-miR-647-001600 | Undetermined | | Undetermined | |
| E6 | hsa-miR-648-001601 | Undetermined | | Undetermined | |
| E7 | hsa-miR-649-001602 | Undetermined | | Undetermined | |
| E8 | hsa-miR-650-001603 | Undetermined | | Undetermined | |
| E9 | hsa-miR-661-001606 | 34,27 | 17,67 | 33,45 | 33,45 |
| E10 | hsa-miR-662-001607 | Undetermined | | Undetermined | |
| E11 | RNU48-001006 | 15,06 | | 15,11 | |
| E12 | hsa-miR-571-001613 | 37,46 | 20,86 | 37,87 | 37,87 |
| E13 | hsa-miR-572-001614 | 27,81 | 11,21 | 29,59 | 29,59 |
| E14 | hsa-miR-573-001615 | 38,90 | 22,29 | 39,74 | 39,74 |
| E15 | hsa-miR-575-001617 | Undetermined | | Undetermined | |
| E16 | hsa-miR-578-001619 | Undetermined | | 39,36 | 39,36 |

| | | | | | |
|---|---|---|---|---|---|
| E17 | hsa-miR-580-001621 | 31,63 | 15,02 | 31,52 | 31,52 |
| E18 | hsa-miR-581-001622 | Undetermined | | Undetermined | |
| E19 | hsa-miR-583-001623 | Undetermined | | Undetermined | |
| E20 | hsa-miR-584-001624 | Undetermined | | Undetermined | |
| E21 | hsa-miR-585-001625 | Undetermined | | Undetermined | |
| E22 | rno-miR-29c#-001818 | 30,50 | 13,90 | 29,64 | 29,64 |
| E23 | hsa-miR-766-001986 | 20,34 | 3,73 | 19,20 | 19,20 |
| E24 | hsa-miR-595-001987 | Undetermined | | Undetermined | |
| F1 | hsa-miR-668-001992 | 22,95 | 6,35 | 38,57 | 38,57 |
| F2 | hsa-miR-767-5p-001993 | 26,33 | 9,72 | 27,32 | 27,32 |
| F3 | hsa-miR-767-3p-001995 | 34,47 | 17,86 | Undetermined | |
| F4 | hsa-miR-454#-001996 | 25,16 | 8,56 | 27,01 | 27,01 |
| F5 | hsa-miR-769-5p-001998 | 23,16 | 6,56 | 21,79 | 21,79 |
| F6 | hsa-miR-770-5p-002002 | 22,89 | 6,28 | 36,19 | 36,19 |
| F7 | hsa-miR-769-3p-002003 | 31,88 | 15,28 | 29,70 | 29,70 |
| F8 | hsa-miR-802-002004 | Undetermined | | Undetermined | |
| F9 | hsa-miR-675-002005 | 39,72 | 23,12 | 26,42 | 26,42 |
| F10 | hsa-miR-505#-002087 | 24,56 | 7,96 | 23,33 | 23,33 |
| F11 | hsa-miR-218-1#-002094 | Undetermined | | Undetermined | |
| F12 | hsa-miR-221#-002096 | Undetermined | | Undetermined | |
| F13 | hsa-miR-222#-002097 | Undetermined | | Undetermined | |
| F14 | hsa-miR-223#-002098 | Undetermined | | Undetermined | |
| F15 | hsa-miR-136#-002100 | 24,35 | 7,75 | Undetermined | |
| F16 | hsa-miR-34b-002102 | 33,82 | 17,22 | 27,83 | 27,83 |
| F17 | hsa-miR-185#-002104 | Undetermined | | Undetermined | |
| F18 | hsa-miR-186#-002105 | Undetermined | | Undetermined | |
| F19 | hsa-miR-195#-002107 | Undetermined | | Undetermined | |
| F20 | hsa-miR-30c-1#-002108 | Undetermined | | 37,61 | 37,61 |
| F21 | hsa-miR-30c-2#-002110 | Undetermined | | Undetermined | |
| F22 | hsa-miR-32#-002111 | Undetermined | | Undetermined | |
| F23 | hsa-miR-31#-002113 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| F24 | hsa-miR-130b#-002114 | 24,20 | 7,60 | 27,02 | 27,02 |
| G1 | hsa-miR-26a-2#-002115 | 31,92 | 15,32 | 32,32 | 32,32 |
| G2 | hsa-miR-361-3p-002116 | 35,35 | 18,75 | 30,94 | 30,94 |
| G3 | hsa-let-7g#-002118 | 32,64 | 16,04 | 33,69 | 33,69 |
| G4 | hsa-miR-302b#-002119 | Undetermined | | Undetermined | |
| G5 | hsa-miR-302d#-002120 | Undetermined | | Undetermined | |
| G6 | hsa-miR-367#-002121 | Undetermined | | Undetermined | |
| G7 | hsa-miR-374a#-002125 | 31,59 | 14,99 | 30,57 | 30,57 |
| G8 | hsa-miR-23b#-002126 | 36,75 | 20,15 | 35,75 | 35,75 |
| G9 | hsa-miR-376a#-002127 | 29,57 | 12,97 | Undetermined | |
| G10 | hsa-miR-377#-002128 | 27,05 | 10,45 | 35,33 | 35,33 |
| G11 | ath-miR159a-000338 | Undetermined | | Undetermined | |
| G12 | hsa-miR-30b#-002129 | Undetermined | | Undetermined | |
| G13 | hsa-miR-122#-002130 | Undetermined | | Undetermined | |
| G14 | hsa-miR-130a#-002131 | Undetermined | | Undetermined | |
| G15 | hsa-miR-132#-002132 | 34,35 | 17,75 | 35,12 | 35,12 |
| G16 | hsa-miR-148a#-002134 | 35,52 | 18,91 | Undetermined | |
| G17 | hsa-miR-33a-002135 | 36,71 | 20,11 | 38,71 | 38,71 |
| G18 | hsa-miR-33a#-002136 | 27,23 | 10,63 | 28,19 | 28,19 |
| G19 | hsa-miR-92a-1#-002137 | 25,62 | 9,01 | 28,19 | 28,19 |
| G20 | hsa-miR-92a-2#-002138 | Undetermined | | Undetermined | |
| G21 | hsa-miR-93#-002139 | 18,51 | 1,90 | 18,43 | 18,43 |
| G22 | hsa-miR-96#-002140 | Undetermined | | Undetermined | |
| G23 | hsa-miR-99a#-002141 | 31,31 | 14,70 | 26,32 | 26,32 |
| G24 | hsa-miR-100#-002142 | Undetermined | | 32,15 | 32,15 |
| H1 | hsa-miR-101#-002143 | Undetermined | | Undetermined | |
| H2 | hsa-miR-138-2#-002144 | 28,49 | 11,88 | 28,66 | 28,66 |
| H3 | hsa-miR-141#-002145 | Undetermined | | Undetermined | |
| H4 | hsa-miR-143#-002146 | Undetermined | | Undetermined | |
| H5 | hsa-miR-144#-002148 | Undetermined | | 33,21 | 33,21 |
| H6 | hsa-miR-145#-002149 | 30,48 | 13,88 | 27,92 | 27,92 |

| | | | | | |
|---|---|---|---|---|---|
| H7 | hsa-miR-920-002150 | Undetermined | | Undetermined | |
| H8 | hsa-miR-921-002151 | Undetermined | | Undetermined | |
| H9 | hsa-miR-922-002152 | 31,62 | 15,01 | 33,82 | 33,82 |
| H10 | hsa-miR-924-002154 | Undetermined | | Undetermined | |
| H11 | hsa-miR-337-3p-002157 | 30,00 | 13,40 | Undetermined | |
| H12 | hsa-miR-125b-2#-002158 | 36,55 | 19,95 | 28,12 | 28,12 |
| H13 | hsa-miR-135b#-002159 | 24,62 | 8,02 | Undetermined | |
| H14 | hsa-miR-148b#-002160 | 27,48 | 10,88 | 26,40 | 26,40 |
| H15 | hsa-miR-146a#-002163 | Undetermined | | Undetermined | |
| H16 | hsa-miR-149#-002164 | 35,50 | 18,89 | 33,13 | 33,13 |
| H17 | hsa-miR-29b-1#-002165 | 37,58 | 20,98 | 30,61 | 30,61 |
| H18 | hsa-miR-29b-2#-002166 | 31,12 | 14,52 | 29,85 | 29,85 |
| H19 | hsa-miR-105#-002168 | 33,76 | 17,15 | Undetermined | |
| H20 | hsa-miR-106a#-002170 | Undetermined | | Undetermined | |
| H21 | hsa-miR-16-2#-002171 | Undetermined | | Undetermined | |
| H22 | hsa-let-7i#-002172 | Undetermined | | 30,03 | 30,03 |
| H23 | hsa-miR-15b#-002173 | 23,92 | 7,32 | 23,32 | 23,32 |
| H24 | hsa-miR-27b#-002174 | 24,66 | 8,06 | 25,34 | 25,34 |
| I1 | hsa-miR-933-002176 | Undetermined | | Undetermined | |
| I2 | hsa-miR-934-002177 | Undetermined | | Undetermined | |
| I3 | hsa-miR-935-002178 | Undetermined | | 21,45 | 21,45 |
| I4 | hsa-miR-936-002179 | Undetermined | | Undetermined | |
| I5 | hsa-miR-937-002180 | 38,45 | 21,85 | Undetermined | |
| I6 | hsa-miR-938-002181 | Undetermined | | 37,20 | 37,20 |
| I7 | hsa-miR-939-002182 | 26,08 | 9,48 | 27,75 | 27,75 |
| I8 | hsa-miR-941-002183 | 25,26 | 8,65 | 26,08 | 26,08 |
| I9 | hsa-miR-335#-002185 | 26,77 | 10,17 | 30,50 | 30,50 |
| I10 | hsa-miR-942-002187 | 24,35 | 7,75 | 25,21 | 25,21 |
| I11 | hsa-miR-943-002188 | 29,20 | 12,60 | 32,96 | 32,96 |
| I12 | hsa-miR-944-002189 | 37,34 | 20,74 | Undetermined | |
| I13 | hsa-miR-99b#-002196 | 21,29 | 4,69 | 22,32 | 22,32 |

| | | | | | |
|---|---|---|---|---|---|
| I14 | hsa-miR-124#-002197 | 27,85 | 11,24 | 29,42 | 29,42 |
| I15 | hsa-miR-541#-002200 | 31,14 | 14,54 | Undetermined | |
| I16 | hsa-miR-875-5p-002203 | 28,45 | 11,84 | 30,13 | 30,13 |
| I17 | hsa-miR-888#-002213 | Undetermined | | Undetermined | |
| I18 | hsa-miR-892b-002214 | 36,99 | 20,38 | 38,52 | 38,52 |
| I19 | hsa-miR-9#-002231 | 20,87 | 4,26 | 20,36 | 20,36 |
| I20 | hsa-miR-411#-002238 | 25,19 | 8,59 | 35,01 | 35,01 |
| I21 | hsa-miR-378-002243 | 21,58 | 4,98 | 24,06 | 24,06 |
| I22 | hsa-miR-151-3p-002254 | 19,70 | 3,10 | 19,05 | 19,05 |
| I23 | hsa-miR-340#-002259 | 23,49 | 6,89 | 23,13 | 23,13 |
| I24 | hsa-miR-190b-002263 | 28,70 | 12,10 | 26,95 | 26,95 |
| J1 | hsa-miR-545#-002266 | 32,24 | 15,63 | 31,87 | 31,87 |
| J2 | hsa-miR-183#-002270 | 21,83 | 5,22 | 32,28 | 32,28 |
| J3 | hsa-miR-192#-002272 | 31,06 | 14,46 | 31,34 | 31,34 |
| J4 | hsa-miR-200b#-002274 | Undetermined | | Undetermined | |
| J5 | hsa-miR-200c#-002286 | Undetermined | | Undetermined | |
| J6 | hsa-miR-155#-002287 | Undetermined | | Undetermined | |
| J7 | hsa-miR-10a#-002288 | Undetermined | | Undetermined | |
| J8 | hsa-miR-214#-002293 | 37,00 | 20,40 | 23,12 | 23,12 |
| J9 | hsa-miR-218-2#-002294 | 25,11 | 8,50 | 24,26 | 24,26 |
| J10 | hsa-miR-129#-002298 | 30,16 | 13,56 | 26,14 | 26,14 |
| J11 | hsa-miR-22#-002301 | 30,84 | 14,24 | 28,17 | 28,17 |
| J12 | hsa-miR-425#-002302 | 22,44 | 5,83 | 22,17 | 22,17 |
| J13 | hsa-miR-30d#-002305 | 26,62 | 10,02 | 26,09 | 26,09 |
| J14 | hsa-let-7a#-002307 | Undetermined | | 35,91 | 35,91 |
| J15 | hsa-miR-424#-002309 | 25,34 | 8,73 | 24,99 | 24,99 |
| J16 | hsa-miR-18b#-002310 | Undetermined | | Undetermined | |
| J17 | hsa-miR-20b#-002311 | 39,12 | 22,51 | 30,00 | 30,00 |
| J18 | hsa-miR-431#-002312 | 27,71 | 11,10 | Undetermined | |
| J19 | hsa-miR-7-2#-002314 | 24,12 | 7,52 | 30,83 | 30,83 |
| J20 | hsa-miR-10b#-002315 | 22,81 | 6,20 | 20,43 | 20,43 |

| | | | | | |
|---|---|---|---|---|---|
| J21 | hsa-miR-34a#-002316 | 29,80 | 13,19 | 23,52 | 23,52 |
| J22 | hsa-miR-181a-2#-002317 | 22,98 | 6,38 | 20,32 | 20,32 |
| J23 | hsa-miR-744#-002325 | 28,33 | 11,73 | 27,65 | 27,65 |
| J24 | hsa-miR-452#-002330 | Undetermined | | Undetermined | |
| K1 | hsa-miR-409-3p-002332 | 15,35 | -1,25 | 21,51 | 21,51 |
| K2 | hsa-miR-181c#-002333 | 30,88 | 14,28 | 27,25 | 27,25 |
| K3 | hsa-miR-196a#-002336 | Undetermined | | Undetermined | |
| K4 | hsa-miR-483-3p-002339 | Undetermined | | 25,09 | 25,09 |
| K5 | hsa-miR-708#-002342 | 39,32 | 22,72 | 37,20 | 37,20 |
| K6 | hsa-miR-92b#-002343 | 32,16 | 15,56 | 32,28 | 32,28 |
| K7 | hsa-miR-551b#-002346 | 28,96 | 12,36 | 28,20 | 28,20 |
| K8 | hsa-miR-202#-002362 | Undetermined | | Undetermined | |
| K9 | hsa-miR-193b#-002366 | 29,16 | 12,56 | 28,47 | 28,47 |
| K10 | hsa-miR-497#-002368 | Undetermined | | 39,70 | 39,70 |
| K11 | hsa-miR-518e#-002371 | Undetermined | | Undetermined | |
| K12 | hsa-miR-543-002376 | 22,06 | 5,45 | 27,24 | 27,24 |
| K13 | hsa-miR-125b-1#-002378 | 30,17 | 13,57 | 29,05 | 29,05 |
| K14 | hsa-miR-194#-002379 | Undetermined | | Undetermined | |
| K15 | hsa-miR-106b#-002380 | 20,81 | 4,21 | 19,55 | 19,55 |
| K16 | hsa-miR-302a#-002381 | Undetermined | | 39,20 | 39,20 |
| K17 | hsa-miR-519b-3p-002384 | 30,09 | 13,49 | 29,52 | 29,52 |
| K18 | hsa-miR-518f#-002387 | Undetermined | | Undetermined | |
| K19 | hsa-miR-374b#-002391 | 36,31 | 19,71 | 33,03 | 33,03 |
| K20 | hsa-miR-520c-3p-002400 | 27,03 | 10,42 | 26,31 | 26,31 |
| K21 | hsa-let-7b#-002404 | Undetermined | | 39,87 | 39,87 |
| K22 | hsa-let-7c#-002405 | Undetermined | | 29,51 | 29,51 |
| K23 | hsa-let-7e#-002407 | 30,11 | 13,51 | 30,20 | 30,20 |
| K24 | hsa-miR-550-002410 | 28,24 | 11,64 | 27,41 | 27,41 |
| L1 | hsa-miR-593-002411 | Undetermined | | Undetermined | |
| L2 | hsa-let-7f-1#-002417 | Undetermined | | Undetermined | |
| L3 | hsa-let-7f-2#-002418 | Undetermined | | 33,23 | 33,23 |

| | | | | | |
|---|---|---|---|---|---|
| L4 | hsa-miR-15a#-002419 | 26,15 | 9,54 | 28,95 | 28,95 |
| L5 | hsa-miR-16-1#-002420 | 25,59 | 8,99 | 27,31 | 27,31 |
| L6 | hsa-miR-17#-002421 | 26,94 | 10,34 | 27,32 | 27,32 |
| L7 | hsa-miR-18a#-002423 | 22,16 | 5,56 | 24,02 | 24,02 |
| L8 | hsa-miR-19a#-002424 | 32,91 | 16,31 | 34,69 | 34,69 |
| L9 | hsa-miR-19b-1#-002425 | 21,94 | 5,33 | 22,63 | 22,63 |
| L10 | hsa-miR-625#-002432 | 21,95 | 5,35 | 24,76 | 24,76 |
| L11 | hsa-miR-628-3p-002434 | 27,28 | 10,68 | 26,51 | 26,51 |
| L12 | hsa-miR-20a#-002437 | 23,66 | 7,06 | 23,61 | 23,61 |
| L13 | hsa-miR-21#-002438 | 31,88 | 15,27 | 30,77 | 30,77 |
| L14 | hsa-miR-23a#-002439 | 35,09 | 18,49 | 33,78 | 33,78 |
| L15 | hsa-miR-24-1#-002440 | 32,63 | 16,02 | 37,17 | 37,17 |
| L16 | hsa-miR-24-2#-002441 | 30,40 | 13,80 | 26,44 | 26,44 |
| L17 | hsa-miR-25#-002442 | 23,97 | 7,37 | 24,36 | 24,36 |
| L18 | hsa-miR-26a-1#-002443 | 27,97 | 11,37 | 27,98 | 27,98 |
| L19 | hsa-miR-26b#-002444 | 28,59 | 11,98 | 28,05 | 28,05 |
| L20 | hsa-miR-27a#-002445 | 27,59 | 10,98 | 26,41 | 26,41 |
| L21 | hsa-miR-29a#-002447 | 33,93 | 17,33 | 28,65 | 28,65 |
| L22 | hsa-miR-151-5P-002642 | 23,44 | 6,84 | 21,72 | 21,72 |
| L23 | hsa-miR-765-002643 | 33,75 | 17,15 | 31,52 | 31,52 |
| L24 | hsa-miR-338-5P-002658 | 33,05 | 16,44 | 28,79 | 28,79 |
| M1 | hsa-miR-620-002672 | Undetermined | | Undetermined | |
| M2 | hsa-miR-577-002675 | 27,13 | 10,53 | 27,86 | 27,86 |
| M3 | hsa-miR-144-002676 | Undetermined | | Undetermined | |
| M4 | hsa-miR-590-3P-002677 | 25,80 | 9,20 | 25,52 | 25,52 |
| M5 | hsa-miR-191#-002678 | 25,77 | 9,17 | 25,25 | 25,25 |
| M6 | hsa-miR-665-002681 | 29,73 | 13,13 | Undetermined | |
| M7 | hsa-miR-520D-3P-002743 | 27,74 | 11,14 | 28,31 | 28,31 |
| M8 | hsa-miR-1224-3P-002752 | 36,81 | 20,20 | Undetermined | |
| M9 | has-miR-1305-002867 | 31,87 | 15,26 | 31,31 | 31,31 |
| M10 | hsa-miR-513C-002756 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| M11 | hsa-miR-513B-002757 | Undetermined | | Undetermined | |
| M12 | hsa-miR-1226#-002758 | 23,78 | 7,18 | 24,83 | 24,83 |
| M13 | hsa-miR-1236-002761 | 31,51 | 14,91 | 34,17 | 34,17 |
| M14 | hsa-miR-1228#-002763 | Undetermined | | Undetermined | |
| M15 | hsa-miR-1225-3P-002766 | 33,74 | 17,14 | 35,71 | 35,71 |
| M16 | hsa-miR-1233-002768 | 27,49 | 10,89 | 26,56 | 26,56 |
| M17 | hsa-miR-1227-002769 | 23,65 | 7,04 | 25,82 | 25,82 |
| M18 | hsa-miR-1286-002773 | Undetermined | | Undetermined | |
| M19 | hsa-miR-548M-002775 | Undetermined | | Undetermined | |
| M20 | hsa-miR-1179-002776 | 26,64 | 10,04 | 30,74 | 30,74 |
| M21 | hsa-miR-1178-002777 | Undetermined | | Undetermined | |
| M22 | hsa-miR-1205-002778 | Undetermined | | Undetermined | |
| M23 | hsa-miR-1271-002779 | 21,59 | 4,98 | 22,22 | 22,22 |
| M24 | hsa-miR-1201-002781 | 24,79 | 8,19 | 23,50 | 23,50 |
| N1 | hsa-miR-548J-002783 | 36,30 | 19,69 | 36,83 | 36,83 |
| N2 | hsa-miR-1263-002784 | Undetermined | | Undetermined | |
| N3 | hsa-miR-1294-002785 | Undetermined | | Undetermined | |
| N4 | hsa-miR-1269-002789 | Undetermined | | 24,74 | 24,74 |
| N5 | hsa-miR-1265-002790 | Undetermined | | Undetermined | |
| N6 | hsa-miR-1244-002791 | 24,28 | 7,68 | 26,99 | 26,99 |
| N7 | hsa-miR-1303-002792 | 28,71 | 12,11 | 29,73 | 29,73 |
| N8 | hsa-miR-1259-002796 | Undetermined | | Undetermined | |
| N9 | hsa-miR-548P-002798 | Undetermined | | 34,94 | 34,94 |
| N10 | hsa-miR-1264-002799 | Undetermined | | Undetermined | |
| N11 | hsa-miR-1255B-002801 | 31,08 | 14,47 | 29,58 | 29,58 |
| N12 | hsa-miR-1282-002803 | 29,62 | 13,02 | 31,26 | 31,26 |
| N13 | hsa-miR-1255A-002805 | Undetermined | | 32,83 | 32,83 |
| N14 | hsa-miR-1270-002807 | Undetermined | | 25,77 | 25,77 |
| N15 | hsa-miR-1197-002810 | 27,35 | 10,75 | 37,45 | 37,45 |
| N16 | hsa-miR-1324-002815 | Undetermined | | Undetermined | |
| N17 | hsa-miR-548H-002816 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| N18 | hsa-miR-1254-002818 | 24,10 | 7,50 | 24,51 | 24,51 |
| N19 | hsa-miR-548K-002819 | 37,50 | 20,90 | Undetermined | |
| N20 | hsa-miR-1251-002820 | 35,25 | 18,65 | Undetermined | |
| N21 | hsa-miR-1285-002822 | 32,37 | 15,77 | 30,83 | 30,83 |
| N22 | hsa-miR-1245-002823 | Undetermined | | Undetermined | |
| N23 | hsa-miR-1292-002824 | 32,82 | 16,22 | 33,75 | 33,75 |
| N24 | hsa-miR-1301-002827 | 31,89 | 15,28 | 26,86 | 26,86 |
| O1 | hsa-miR-1200-002829 | Undetermined | | Undetermined | |
| O2 | hsa-miR-1182-002830 | Undetermined | | Undetermined | |
| O3 | hsa-miR-1288-002832 | 38,20 | 21,59 | 39,97 | 39,97 |
| O4 | hsa-miR-1291-002838 | 30,00 | 13,39 | 30,40 | 30,40 |
| O5 | hsa-miR-1275-002840 | 21,29 | 4,69 | 21,72 | 21,72 |
| O6 | hsa-miR-1183-002841 | 26,72 | 10,11 | 26,39 | 26,39 |
| O7 | hsa-miR-1184-002842 | Undetermined | | Undetermined | |
| O8 | hsa-miR-1276-002843 | 28,01 | 11,41 | 29,31 | 29,31 |
| O9 | hsa-miR-320B-002844 | 23,85 | 7,24 | 21,66 | 21,66 |
| O10 | hsa-miR-1272-002845 | Undetermined | | 36,61 | 36,61 |
| O11 | hsa-miR-1180-002847 | 21,08 | 4,47 | 22,03 | 22,03 |
| O12 | hsa-miR-1256-002850 | Undetermined | | 38,00 | 38,00 |
| O13 | hsa-miR-1278-002851 | Undetermined | | Undetermined | |
| O14 | hsa-miR-1262-002852 | 36,91 | 20,30 | 32,63 | 32,63 |
| O15 | hsa-miR-1243-002854 | 25,26 | 8,66 | 26,64 | 26,64 |
| O16 | hsa-miR-663B-002857 | 31,98 | 15,38 | 32,65 | 32,65 |
| O17 | hsa-miR-1252-002860 | Undetermined | | Undetermined | |
| O18 | hsa-miR-1298-002861 | Undetermined | | Undetermined | |
| O19 | hsa-miR-1290-002863 | 25,31 | 8,71 | 22,21 | 22,21 |
| O20 | hsa-miR-1249-002868 | 29,22 | 12,61 | 29,56 | 29,56 |
| O21 | hsa-miR-1248-002870 | 31,69 | 15,09 | 30,05 | 30,05 |
| O22 | hsa-miR-1289-002871 | Undetermined | | Undetermined | |
| O23 | hsa-miR-1204-002872 | Undetermined | | Undetermined | |
| O24 | hsa-miR-1826-002873 | Undetermined | | Undetermined | |

| | | | | | |
|---|---|---|---|---|---|
| P1 | hsa-miR-1304-002874 | Undetermined | | Undetermined | |
| P2 | hsa-miR-1203-002877 | Undetermined | | Undetermined | |
| P3 | hsa-miR-1206-002878 | Undetermined | | Undetermined | |
| P4 | hsa-miR-548G-002879 | Undetermined | | Undetermined | |
| P5 | hsa-miR-1208-002880 | 36,79 | 20,18 | 30,00 | 30,00 |
| P6 | hsa-miR-548E-002881 | 37,11 | 20,51 | Undetermined | |
| P7 | hsa-miR-1274A-002883 | 18,80 | 2,19 | 20,19 | 20,19 |
| P8 | hsa-miR-1274B-002884 | 15,88 | -0,72 | 16,45 | 16,45 |
| P9 | hsa-miR-1267-002885 | 26,85 | 10,25 | 26,88 | 26,88 |
| P10 | hsa-miR-1250-002887 | Undetermined | | 35,64 | 35,64 |
| P11 | hsa-miR-548N-002888 | Undetermined | | Undetermined | |
| P12 | hsa-miR-1283-002890 | Undetermined | | Undetermined | |
| P13 | hsa-miR-1247-002893 | 27,82 | 11,22 | 23,69 | 23,69 |
| P14 | hsa-miR-1253-002894 | 30,60 | 14,00 | 32,37 | 32,37 |
| P15 | hsa-miR-720-002895 | 15,40 | -1,21 | 16,34 | 16,34 |
| P16 | hsa-miR-1260-002896 | 24,40 | 7,80 | 25,53 | 25,53 |
| P17 | hsa-miR-664-002897 | 27,22 | 10,61 | 26,32 | 26,32 |
| P18 | hsa-miR-1302-002901 | 39,64 | 23,03 | Undetermined | |
| P19 | hsa-miR-1300-002902 | 30,30 | 13,70 | 30,51 | 30,51 |
| P20 | hsa-miR-1284-002903 | Undetermined | | Undetermined | |
| P21 | hsa-miR-548L-002904 | 35,69 | 19,09 | 33,13 | 33,13 |
| P22 | hsa-miR-1293-002905 | Undetermined | | Undetermined | |
| P23 | hsa-miR-1825-002907 | Undetermined | | Undetermined | |
| P24 | hsa-miR-1296-002908 | 29,12 | 12,51 | 26,80 | 26,80 |