MicroRNAs in melanocyte and melanoma biology

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MicroRNAs in melanocyte and melanoma biology

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Summary

The importance of microRNAs as key molecular components of cellular processes is now being recognized. Recent reports have shown that microRNAs regulate processes as diverse as protein expression and nuclear functions inside cells, and are able to signal extracellularly, delivered via exosomes, to influence cell fate at a distance. The versatility of microRNAs as molecular tools inspires the design of novel strategies to control gene expression, protein stability, DNA repair and chromatin accessibility that may prove very useful for therapeutic approaches due to the extensive manageability of these small molecules. However, we still lack a comprehensive understanding of the microRNA network and its interactions with the other layers of regulatory elements in cellular and extracellular functions. This knowledge may be necessary before we exploit microRNA versatility in therapeutic settings. In order to identify rules of interactions between microRNAs and other regulatory systems, we begin by reviewing microRNA activities in a single cell type: the melanocyte, from development to disease.

Keywords - Melanocytes, Pigmentation, Melanoma, miRNA, miRNA processing
Running Title - MicroRNAs in melanocyte and melanoma biology

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1 - Introduction

First discovered in *C.elegans* in 1993 (Lee et al., 1993), a growing number of microRNAs have been reported ever since in plants and animals. In humans, more than 2500 microRNA are listed by miRBase nowadays (http://www.mirbase.org/cgi-bin/browse.pl?org=hsa). MicroRNAs are short RNA molecules (22-24 nt) encoded by intronic (some, called miRtrons, actually require the splicing machinery for their processing (Berezikov et al., 2007) or intergenic sequences. They are transcribed by RNA polymerase II as larger pri-miRNAs, then processed by the RNase III enzyme Drosha and DGCR8 in the nucleus to yield ~70 nt hairpin precursors, the pre-miRNAs (Krol et al., 2010). Next, they are exported to the cytoplasm by exportin-5 (Melo et al., 2010), where the pre-Mirs are cleaved into mature duplexes (dsRNAs) by a complex including Dicer, Ago2 (argonaute 2) and TRBP (trans-activation-responsive RNA-binding protein) (Chendrimada et al., 2005). One strand of the dsRNAs is inserted into the RNA-inducing silencing complex (RISC), where the

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miRNA plays its role of negative regulator of mRNA stability or translation. This is generally exerted through perfect or imperfect pairing with a "seed" sequence in the 3'UTR or coding region of target transcripts usually corresponding to nt 2-8 of the mature microRNA sequence. The other strand of the duplex (the star * or passenger strand) is usually degraded, but recent data suggest that many passenger microRNA strands also have functions (Yang et al., 2011).

Seed sequences are highly conserved among species and used to categorize different miRNA families (Griffiths-Jones et al., 2008). In many cases base pairing occurs between nucleotides 2–8, the seed region of the miRNA; however, a recent unbiased technique for sequencing miRNA–mRNA duplex complexes, named CLASH, identified at least four additional non-canonical binding sequences or location motifs. Moreover, more than 35% of the binding events were independent of nucleotides 2–8, highlighting the complexity in miRNA target identification (Helwak et al., 2013). Besides the well known functions as negative regulators of mRNA translation through the RISC complex, non-canonical microRNA and star-microRNA functions are continously reported. These include functions exerted in the cytoplasm, where microRNAs (especially paralogues, or derived from pseudogenes) can function as microRNA sponges (Hansen et al., 2013) or as competing endogenous RNA, ceRNA (Tay et al., 2014) thus effectively exerting target protection of their mRNA targets from „canonical" microRNAs. MicroRNAs not only bind to untranslated regions (UTRs) but also to coding sequences (Fang and Rajewsky, 2011; Tay et al., 2008); and 5' UTRs. Moreover, some miRNAs have functions in translational promotion; for example, miR-10 is involved in enhancing mRNA translation of a ribosomal protein by binding to its 5'-UTR (Orom et al., 2008).

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Further, nuclear functions of microRNAs have been reported. Recent studies indicate that mature miRNA can be imported into the nucleus and repress ncRNAs. MALAT1, a long ncRNA is a target of miR-9 in the nucleus (Leucci et al., 2013). (Pasquinelli and Ruvkun, 2002) suggested that miRNAs can bind to pre-mRNAs and DNA to facilitate alternative splicing, and form RNA–DNA duplexes with variable consequences on transcription. DNA repair can also be controlled by small ncRNAs acting at the site of DNA lesions. In a recent report, small ncRNAs, with sequences complementary to damaged DNA sites, were identified in DNA damaged cells and termed DNA damage response RNAs (DDRNAs) (Francia et al., 2012). Their role is

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signaling the presence of DNA damage in the cell. As they are processed through the classical microRNA biogenesis machine and have a 20-24 nt length, they highly resemble microRNAs. Although it's still not clear whether they correspond to a class of microRNAs (d'Adda di Fagagna, 2014), the regulation of their expression is definitely different from that of classical microRNAs.

The regulation of microRNA levels and functions is also complex: besides classical cis- and trans-acting regulatory elements driving expression of single or clustered microRNAs like for any gene, for intragenic microRNAs the genomic location often determines their regulation. For microRNAs subjected to nuclear regulation, the processing of pri-miRNA to pre-miRNA is determined by direct and indirect interactions with proteins, including competition between microprocessors and spliceosome for a primary transcript that contains a segment of a pre-miRNA at its exon–intron junction (Ramalingam et al., 2014). Cytosolic regulation, takes place when different factors affect pre-miRNA maturation (and degradation). Recently, the hippo pathway effector, Yap1 was shown to affect the ability of the microprocessor to produce pre-miRs in relation with low cell density (Mori et al., 2014).

MicroRNAs are also subjected to posttranscriptional modifications. There are two types of miRNA modifications: nucleotide modification and nucleotide addition. The former is an epigenetic event: adenosine (A) deamination results in conversion to inosine (I). Inosine has similar properties as guanosine (G) in base pairing, thus it can alter the pre-miRNA structure and mature sequences by affecting both miRNA processing and target recognition (Kawahara et al., 2007a; Kawahara et al., 2007b). For instance, A-to-I editing inside the seed sequence has been observed in some miRNAs, such as miR-151, miR-376a, miR-376b, and miR-368 (Garcia-Lopez et al., 2013; Kawahara et al., 2007b). Apart from A-to-I editing, mature miRNAs can be modified at the 3'-end through uridylation or adenylation (Chiang et al., 2010; Katoh et al., 2009). This type of editing has been found in many miRNAs (Kawahara et al., 2007b; Luciano et al., 2004) and may affect miRNA biogenesis.

Several mature miRNAs have size variants, termed isomiRs. They are present in divergent species (Bizuayehu et al., 2012; Humphreys et al., 2012; Lee et al., 1993; Li et al., 2011). The origin of isomiRs is still not fully understood. Different mechanisms have been proposed, including degradation or imprecise cleavage of pre-miRNA during processing. However, the occurrence of isomiRs is likely

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nonrandom. Although the random degradation of mature miRNAs by nucleases cannot be excluded, the differential expression patterns and the observed target differences (Bizuayehu et al., 2012; Humphreys et al., 2012; Wei et al., 2012) suggest that biosynthesis of isomiRs is a regulated process.

2 - Regulatory pathways in melanocyte development and disease

The process of melanocyte development and pigmentation is mostly conserved among vertebrates, with important differences that are covered by excellent reviews (Hofreiter and Schoneberg, 2010; Kelsh, 2004; Mills and Patterson, 2009).

In mammals, melanocyte precursors, termed melanoblasts, migrate along a dorso-lateral pathway beneath the ectoderm, and colonize skin and hair follicles. There, they differentiate into mature melanocytes and begin production of melanin, which is synthesized within specialized organelles known as melanosomes. Thus melanocytes are responsible for the extensive and complex patterns of pigmentation seen in vertebrates, and provide protection against solar UV radiation, as melanocytes respond to environmental conditions by increasing pigment production (Miyamura et al., 2007). Importantly melanocytes are equipped with specialized cytoskeleton for complex sub-cellular trafficking of melanosomes which also responds rapidly to light (reviewed in (Ohbayashi and Fukuda, 2012). Their lineage in the adult is maintained through the establishment, and ad hoc differentiation of adult melanocyte stem cells within hair follicles in mammals and in different locations in other vertebrates (Osawa et al., 2005).

Defects in melanocyte development and function are associated with a variety of human diseases and disorders, whereas the cancerous growth of melanocytes results in melanoma, an aggressive cancer with a high mortality rate. All these conditions are genetically heterogeneous, and identification of the genetic causes of these diseases have revealed genes and pathways that function in normal development of melanocytes. Today, more than 120 genes regulating melanocyte development and migration, formation of the melanosome and pigmentation have been identified (Baxter et al., 2009).

MITF, PAX3, and SOX10 transcription factors form the core of melanocyte differentiation program and have important roles in melanocyte development, specification, migration, survival and differentiation. MIcrophthalmia-associated Transcription Factor (MITF) plays a central role in melanocyte development and

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function. It is required for melanocyte differentiation, survival, generation of melanogenic proteins, and is associated with melanoma progression (Goding, 2000; Hodgkinson et al., 1993). MITF regulates several cellular functions in melanocytes, including cell survival (McGill et al., 2002), cell motility and cell cycle progression (Carreira et al., 2005) and response to the environment (Sato-Jin et al., 2008). PAX3 (paired box 3) activates transcription of MITF (Watanabe et al., 1998) and plays a crucial role in maintaining melanocyte stem cells (Lang et al., 2005). SRY-box containing gene 10 (SOX10) regulates specification of neural crest-derived melanocytes, neurons, and glia (Mollaaghababa and Pavan, 2003). SOX10 strongly activates MITF and regulates expression of melanogenic enzymes (Hou et al., 2006). UV irradiation results in activation of multiple cellular processes in melanocytes, including the Melanocortin1 receptor pathway, which stimulates cAMP (cyclicAMP) formation and subsequently initiates melanogenesis, stimulates proliferation, inhibits apoptosis, and enhances DNA repair of the direct mutagenic effects of UV radiation on DNA (Abdel-Malek et al., 2008).

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3 - MicroRNAs in melanocyte biology

Besides the approximately 100 genes known to affect melanocyte development, there is a growing number of microRNAs, which target genes or pathways involved in melanocyte biology. Many of these have been identified through studies in melanoma cells or samples, and for the vast majority, their role(s), if any, in melanocyte development or biology is still unknown. Here we focus on those microRNAs that have been shown to play a role in physiological conditions, in addition to, or regardless from, a role in melanoma. The first evidence of a role of microRNAs in melanocyte biology came from the observation that Dicer is required for survival of differentiating neural crest cells (Zehir et al., 2010). However, until today, only a few microRNAs regulating neural crest migration, specification and differentiation have been reported. Eberhart et al. (Eberhart et al., 2008), showed that miR-140, affects cranial neural crest cell dispersion. Further studies have identified several miRNAs playing different roles in neural crest development (Amaral and Mattick, 2008; Cordes and Srivastava, 2009; Ivey and Srivastava, 2010; Subramanyam and Blelloch, 2011; Xin et al., 2009). MiR-143 and miR-145 have been shown to direct exit from pluripotency by targeting Klf4 (Krüppel-like factor 4), Sox2, and Oct4 (Octamer

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binding transcription factor 4) (Xu et al., 2009). Conditional deletion of Dicer from neural crest lineage does not prevent induction and migration of neural crest, but impairs migration and patterning of cardiac precursors, resulting in a variety of cardiovascular abnormalities (Huang et al., 2010b; Nie et al., 2011; Zehir et al., 2010). Dicer-induced phenotypes are, at least in part, mediated by miR-21 and miR-181a, which in turn, affects the MEK/ERK signaling pathway (Huang et al., 2010a; Huang et al., 2010b).

Using miRNA profiling on mouse melanocytes after consecutive treatments involving forskolin and solar-simulated UV (ssUV) irradiation, (Dynoodt et al., 2013) identified sixteen miRNAs differentially expressed in treated melan-a cells versus untreated cells. Overexpression or downregulation of miR-145 in melan-a cells revealed reduced or increased expression of Sox9, Mitf, Tyr, Trp1, Myo5a, Rab27a, and Fscn1, respectively, placing miR145 in a central role for regulation of melanogenesis in response to external stimuli. Recently miR-125b was identified as a potent regulator of steady-state melanogenesis. (Kim et al., 2014) found that the expression of miR-125b is inversely related to pigment levels. Targets of miR125b are Tyr and DCT, two key enzymes in the production of melanin. In darkly pigmented cells the *MIR125B-1* promoter is hypermethylated. Although a downregulation of miR-125b has been reported in melanoma (see below), no link to pigmentation was observed in melanoma cell lines or samples.

An extensive network of microRNAs centered around MITF in melanocyte differentiation, is emerging through the efforts of different labs. (Levy et al., 2010) reported that MITF regulated DICER transcription in melanocytes. It binds and activates a conserved regulatory element upstream of DICER's transcriptional start site upon melanocyte differentiation, thus allowing maturation of specific microRNAs involved in melanocyte survival. Targeted KO of DICER is lethal to melanocytes, similar to MITF deprivation (Goding, 2000), partly via DICER-dependent processing of the pre-miRNA-17- 92 cluster targeting BIM, a known proapoptotic regulator of melanocyte survival (Levy et al., 2010). Dicer is strongly upregulated during melanocyte differentiation. In turn, MITF was suggested to be a target of miR-25 in melanocytes. Microarray screening of miRNAs in the skin of alpacas with pigmented vs. white coat color identified differentially expressed miR-25, conserved binding sites of which are found in the 3'UTR of MITF from human, rat, mouse and dog. Overexpression of miR-25 in cultured melanocytes, indeed, reduced MITF mRNA

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and protein levels as well as the expression of the MITF-regulated enzymes tyrosinase and tyrosinase-related protein (Zhu Z, 2010). In 2012, (Dong et al., 2012) showed that miR-137 mediated down-regulation of MITF in a transgenic mouse model expressing multiple copies of pre-MirR137 under a CMV (cytomegalo virus) promoter. Transgenic mice developed coat color changes related to the levels of miR137 expression. Molecular analyses of the transgenic mice (3 copies of pre-MirR137 under CMV promoter) showed decreased expression of MITF (only at the protein level!) and of its downstream genes, including TYR (tyrosinase), TYRP1 (tyrosinase-related protein 1), and TYRP2 (tyrosinase-related protein 2). As for miR145 (see above) melanogenesis altered by miR-137 is distinct from that affected by UV radiation. A response to UV however is present also in transgenic mice and is mediated by a-MSH (alpha melanocyte-stimulating hormone. Alpha-MSH inhibits the expression of endogenous miR-137 (Bemis et al., 2008), activates MITF, and produces more melanin in skins to partially compensate for the melanin reduction induced by exogenous miR-137. Thus, a complex regulatory loop is established that compensate changes in miR137 expression. The involvement of miR-340 in the regulation of MITF warrants further investigation (Goswami et al., 2010) retracted in 2014); however the same microRNA was reported to regulate UVB-Induced dendrite formation through the regulation of RhoA expression in melanocytes (Jian et al., 2014). In response to skin penetration by UV rays and subsequent DNA damage, melanocytes form dendrites, which are specialized cell structures that transport melanosomes to their tips for transfer to the surrounding keratinocytes and protect them from UV irradiation (Eller et al., 1997). UV-induced DNA damage generates thymidine dinucleotide fragments, which induce melanogenesis and cause the melanocyte to produce melanosomes (Yamaguchi et al., 2007). (Jian et al., 2014) show that miR-340 was upregulated 4.8-fold in pigmented cells treated with UVB irradiation. Overexpression of miR-340 significantly repressed RhoA protein expression, increased the number and total length of dendrites per cell, and promoted melanosome aggregation in dendritic tips of melanocytes.

Conversely, in the retinal pigmented epithelium (RPE), MITF promotes differentiation by regulating microRNAs-204/211 expression. Mir-204/211 transfection in de-differentiated RPE cultures is sufficient to induce an epithelial differentiation (Adijanto et al., 2012).

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These results indicate the existence of a crosstalk between the miRNAs and pigment-specific master regulators, which is important for a proper balance of gene expression in melanocytes. There is still limited information available about the functions of additional individual miRNAs in melanocyte biology. Several groups reported results on miRNA profiling in normal epidermal melanocytes. For example, microarray analysis showed that miR-192 and miR-194 are being expressed at high levels in the normal epidermal melanocytes (Caramuta et al., 2010).

Deep sequencing of epidermal melanocyte libraries revealed relatively high levels of expression for several miRNAs, including the members of the let-7 (let-7a-i), miR-219a, miR-320a and miR-378 families (Stark et al., 2010). However, the role of these miRNAs and their targets in pigment cells requires further investigations.

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As Dicer, a key component of the miRNA processing machinery, is a target of the melanocyte-specific master regulator MITF, this suggests that miRNAs are important components of cell type-specific transcriptional programs. In turn, MITF is itself a target of individual miRNAs (mir-137, miR-125b and miR-25, possibly miR-340), establishing a negative feedback mechanisms that controls MITF expression in differentiating cells (reviewed in (Botchkareva, 2012).

MicroRNAs have been involved in the control of skin pigmentation also in fish (Yan et al., 2013). Thirteen miRNAs were found differentially expressed between red and white skin carps (Cyprinus carpio) and 10 of them showed the same behavior in red/white tilapia. The analysis of miRNA spatial and temporal expression patterns suggests that miR-429 is a potential regulator of skin pigmentation. miR-429 silencing in red-skin carp resulted in de-pigmentation. Bioinformatics analysis and a luciferase reporter assay show that miR-429 directly regulates expression of Foxd3 by targeting its 3'- UTR. MiR-429 silencing leads to a substantial increase in the expression of Foxd3 in vivo, thereby repressing the transcription of Mitf and its downstream genes, such as Tyr, Tyrp1 or Tyrp2. Foxd3 has been shown to have a function in the specification of various downstream neural crest derivatives. miR-429-mediated pigmentation is distinct from a-MSH mediated pigmentation upon UV radiation: however, the authors found that UV radiation significantly upregulates the level of miR-429 expression in the melanocyte, providing support for convergence of pro-pigmentation signals on miR-429. The changes in miR-429 expression could be

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detected as early as 1 hour after UV treatment, thus showing that miRNA regulation occurs earlier than most gene transcription responses leading to a significant reduction in Foxd3 expression and a marked increase in MITF expression at mRNA and protein levels.

MiR-429 is a member of the miR-8 family. In Drosophila miR-8 is required for proper spatial patterning of pigment on adult female abdomens. Loss of miR-8 in the developing cuticle results in cell-autonomous loss of pigmentation (Kennell et al., 2012). Foxd3 is one of the earliest molecular markers of the neural crest lineage; Foxd3 can also control the lineage choice between neural or glial and pigment cells by repressing MITF during the early phase of neural crest migration. In this study, miR-429 expression was shown to begin at the gastrula stage, which leads to a gradual decrease in expression of Foxd3. This progressive reduction of MITF repression by foxD3 could contribute to the generation of pigment cells.
Until today, no data in human or mice on the effect of miR-429 on pigmentation is available.

4 - MicroRNAs in melanoma and melanoma-subtype specific microRNAs

The role of specific miRNAs in melanoma development and progression was addressed in several recent reviews (Leibowitz-Amit et al., 2012; Luo et al., 2014; Sun et al., 2014; Voller et al., 2013a). Therefore, in this review only details on **recent new findings** are given. Due to fast progresses in the field, this list does not claim to be complete.
In a study by Greenberg et al. the miR17/92 complex was addressed (Greenberg et al., 2014). In a previous study, they revealed upregulation of miR-17, which directly targets ADAR1(adenosine deaminase acting on RNA 1) and modulates melanoma aggressiveness (Nemlich et al., 2013). In the new study the group focused on miR-20a, belonging to the same complex and carrying an identical seed sequence as miR-17. MiR-20a was also shown to be induced in melanoma (Couts et al., 2013; Levati et al., 2009). Interestingly and despite the identical seed sequence, the group observed a different pattern of target genes for the individual miRs resulting also in different functional effect. Two non-seed nucleotides are different between the two miRs in their mature form and the authors speculate that this difference obviously results in differential target sequence recognition. This finding supports several other

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3 studies showing differential regulation of target genes by one family of miRs and
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5 illustrates that miRs out of one family surely not have to be redundant.

As described above, miR-20a was found to be differentially expressed comparing melanocytes to melanoma cell lines. Now Saleiban et al. revealed that miR-20b is strongly downregulated in melanoma and targets proteinase-activated receptor-1 (PAR-1) when re-expressed in the cells (Saleiban et al., 2014).

Downregulation of miR-125b was addressed previously in three studies. There, direct regulation of c-Jun expression (Kappelmann et al., 2013) and an influence on cell proliferation, migration and senescence was observed (Nyholm et al., 2014). In a new study by Zhang et al., the serine/threonine kinase mixed lineage kinase 3 (MLK3) was shown to be a direct target of miR-125b (Zhang et al., 2014a). Interestingly, no link to pigmentation was observed in melanoma as recently described by Kim et al. for miR-125B in melanocytes (Kim et al., 2014).

Li et al. reported that miR-143 was significantly lower expressed in melanoma tissues compared to normal skin (Li et al., 2014c), which confirms a study by Segura et al. (Segura et al., 2010). Segura et al. described miR-143 as one of the miRs predicting longer survival when being highly expressed. In addition, Li et al. revealed that miR-143 regulates expression of Syndecan-1 and showed that re-expression of miR-143 resulted in inhibition of proliferation and induction of apoptosis.

miR-203 was described in previous studies to play a role as suppressor of tumorigenesis in melanoma by influencing cell cycle arrest and senescence (Noguchi et al., 2012). Further, several experiments defining deregulated miRs in melanoma found miR-203 downregulated in melanoma (Chen et al., 2010; Philippidou et al., 2010; Xu et al., 2012). Bu and Yang could now identify Versican as an additional target of miR-203 (Bu and Yang, 2014). They revealed effects of miR-203 on melanoma migration via directly targeting Versican.

Deregulation of miR-206 was observed in several kinds of cancer. Georgantas et al. described a downregulation of miR-206 in melanoma tissue and cell lines and further revealed that miR-206 directly targets CDK4 (cyclin-dependent kinase 4), Cyclin D1 and Cyclin C. Thereby, miR-206 was shown to induce a G1 arrest after re-expression in melanoma cells (Georgantas et al., 2014).

Two new studies focused on miR-218 (Guo et al., 2014a; Wei et al., 2014), which was also addressed in studies on melanoma subtypes (Chan et al., 2011; Huang et al., 2010a) (see the following section). All studies revealed strong deregulation of

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miR-218 expression, furthermore, CIP2A (cancerous inhibitor of PP2A) and BMI1 (BMI1 polycomb ring finger oncogene) but also MITF were demonstrated to be direct target genes of miR-218 having a strong impact on melanoma cell proliferation and invasion.

Poenitzsch et al. presented miR-340 as a regulator of RAS-RAF-MAPK signaling by modulating several proteins out of this signaling pathway (Poenitzsch Strong et al., 2014). Recently, miR-340 was already described to regulate MITF in melanoma suggesting miR-340 to be an important regulator in melanoma development (Goswami et al., 2010).

Another commonly found aspect comparing different types of cancer is a kind of cell-type specificity of miR action. Target genes of one miR, which were clearly demonstrated to be a direct target in one type of cancer are not regulated in other types. One potential explanation is addressed in a recent study by Pedace et al (Pedace et al., 2014). They revealed changes in the 3'UTR of CDK4, which interfere with miR target sites and lead to an increased risk of melanoma (Pedace et al., 2014). Similar finding were reported by other groups. (Godshalk et al., 2011), for example, described changes in the 3'UTR of KIT resulting in an increase risk for acral melanoma. Of course, mutations in 3'UTR cannot be the only reason for the cancer-type specific regulation of miR/target gene interaction. Differential expression of miRNA processing proteins (see section 5- microRNA processing defects) could also be implicated, in addition to other potential mechanisms that were addressed in a recent review (Palanichamy and Rao, 2014).

In 2010, we defined miRs being involved in melanoma development and progression as "melano-mirs" (Mueller and Bosserhoff, 2010). In recent years this field has further evolved and gathered complexity. Until now, we further gained detailed insight into miRs of importance in melanoma. Still, we have to mainly focus on one miR one gene interaction at a time, as the field is very complicated. New knowledge will finally result in the understanding of networks of miRs and target genes (see section 6). Interestingly, characterization of tissue of origin and, as illustrated in the following paragraphs, subtype specificity is possible by miRs, proving that stable and tightly regulated networks are common.

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Five studies addressed **subtype specific changes** in melanoma in detail (Chan et al., 2011; Huang et al., 2010a; Kozubek et al., 2013; Poliseno et al., 2012; Stark et al., 2010). Most commonly melanoma is subdivided into 4 main subtypes with superficial spreading melanoma (SSM, 70%) and nodular melanoma (NM, 20%) being the most common types. Three of the studies analyzed tissues and defined the subtype of primary melanomas. In the study of Kozubek et al., 4 SSM and one NM were analyzed but due to low numbers these were all included in the group of primary melanomas and no further detailed analysis was performed based on the different subtypes. Poliseno et al. set up a large study including SSM, NM and further congenital nevi. They revealed 126 miRNAs being differentially expressed comparing SSM and NM and concluded that the subtypes are clearly based on distinct molecular alterations. This is interesting as a linear progression from SSM to NM is speculated which would be ruled out by these findings. Most importantly, the study revealed that specific downregulation of 6 of the miR in SSM is associated with loss of the respective genomic locus further supporting the theory of different molecular subtypes (Poliseno et al., 2012). Chan et al. evaluated acral versus non acral melanoma and observed differential expression of miR-142-3p, miR-486, miR-214, miR-218, miR-362, miR-650 and miR-31 (Chan et al., 2011). Further, subdivision based on the mutations in the tumors was performed in this study. Here, 13 samples carrying BRAF mutations were compared to 8 tumors with NRAS_Q61 mutations. Interestingly, no significant differences in the expression of microRNAs could be observed. Stark et al. demonstrated that the miR expression (using all miRs detected in their study, including new miRs) resulted in a good separation of different histological subtypes of melanoma like mucosal, acral. uveal and skin melanoma (Stark et al., 2010).

In the study of Hwang et al. (Hwang et al., 2014) the 5 cell lines analyzed were subdivided with regards to the classification by Hoek and Goding (Hoek and Goding, 2010) into “proliferative” and “invasive” melanoma cell lines. They were able to define a set of miRNAs discriminating between both groups. Interestingly, they revealed that HIF1alpha and hypoxia were the regulators of the upregulation of miR-210, -218, -224 and -452 in the cell lines belonging to the “invasive” pathway. This study thereby further helps to characterize the two states of melanoma cells defined by Hoek and Goding and links the “invasive” pathway to hypoxia.

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As intensively addressed in the review by (Leibowitz-Amit et al., 2012) the low interexperimental concordance is a major problem and needs to be addressed in further studies. Potentially, the new tools provided by next generation sequencing with the ability to better quantify miRNA molecule numbers and to discriminate between wild type and mutated miRs (which cannot be clearly determined in arrays or RT-PCRs) will give further insights. Kozubek et al. (Kozubek et al., 2013) clearly demonstrated higher and more precise levels of detection of miRs by NGS compared to RT-PCR.

Earlier studies indicated that **variations in the sequence** of miR precursors can be commonly found in cancer cell lines (Diederichs and Haber, 2006). Kozubek et al. identified 429 unique sequences in their NGS of melanoma samples and suggested that they are new miRNAs by defined criteria (Kozubek et al., 2013). Some of these are significantly deregulated in their expression. Stark et al. were able to define 279 novel candidate miRs and validated expression of one of the new miRs (Stark et al., 2010).

Further, isomiRs were defined by Morin et al. as miR sequences with variations compared to the reference miRNA sequence (Morin et al., 2008). IsomiRs can be of several origins: they can be trimming variants due to slight variations of the Drosha and/or Dicer cleavage site, generated by nucleotide addition (Wyman et al., 2011), by nucleotide substitution as by Adenosine-deaminase but also be derived from genomic mutations. Kozubek et al. recognized in their study that 6 out of 10 analyzed isomiRs were not recognized by miRBase (Kozubek et al., 2013).

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Another interesting topic is the field of miR* (*read miRNA star*). Both strands of miR duplexes are produced in equal amounts by enzymatic processing, but their accumulation is mainly asymmetric. Initially annotated by the first miR sequencing approaches one strand of a processed pre-miR is referred as miR and the second strand is known as “passenger strand” or miR*. The mechanism of miR strand selection and RNA-induced silencing complex (RISC) loading are still mainly unclear. In melanoma, the relative ratio of miR to miR* varies from 0.03:1 to 242,000:1 defined in a study of Stark et al. by deep sequencing (Stark et al., 2010), revealing that 21 miRs* are much strongly expressed compared to the respective miR and 23

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different miRs* are equally expressed also in melanoma. As miRs* are only rarely addressed in studies, we have to wait for new NGS reports. These will determine whether selection for miR or miR* changes during development or during the progression of the disease or whether a defined pattern is specific for a certain tumor or tumor subtype.

ceRNA in melanoma were addressed in 2011 by a very interesting study of Karreth et al.(Karreth et al., 2011). No further studies are available until today in melanoma, but in other kinds of cancer additional functions of ceRNAs were determined (e.g.(Li et al., 2014a; Yang et al., 2014). Possibly these and other studies are transferable to melanoma and detailed analyses of these and further pathways are of importance.

Long noncoding RNAs (lncRNAs) like HotAir, UCA1, Malat-1, Anril, Llme, SPRY4-IT1 or BANCR have been linked to melanoma development in several studies (Khaitan et al., 2011; Li et al., 2014b; Pasmant et al., 2007; Tang et al., 2013; Tian et al., 2014; Wu et al., 2013). These non protein-coding transcripts (longer than 200 nucleotides) were shown to be implicated in tumorigenesis; however their functions and downstream mechanisms are still largely unknown.

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5 - MicroRNA processing defects

Next to deregulated miRNAs in physiological and pathophysiological processes, the role of miRNA processing and the implicated proteins came into focus. Several basic questions related to miR processing still needs to be addressed. The role of the individual AGOs and a putative specificity for certain miRNAs, mRNAs or miRNA/mRNA combinations is debated. Further the role of specific molecules in the processing like AGO2 and DICER is still under analysis. Here, models in deficient mice and additional studies revealed that processing independent of AGO2 or DICER, respectively, is possible but specific mRNA regulation is missing. Interestingly, DGCR8 (DiGeorge Syndrome Critical Region 8) was recently linked to stem cell phenotype, cellular reprogramming and differentiation in keratinocytes further supporting a role of miRNA processing proteins in cancer development (Chakravarti et al., 2014). TARBP2, the double-stranded RNA-binding protein involved in miR processing, was shown to be overexpressed in breast cancer and

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revealed to promote cell invasion and colonization (Goodarzi et al., 2014). In melanoma, the following studies revealed an impact of miR processing on tumorigenesis. Levy et al. revealed an important role of DICER in melanocytes showing that a target knockdown of DICER in melanocytes is lethal for melanocytes (Levy et al., 2010). In melanoma, DICER expression was analyzed in detail, in part resulting in divergent findings. In studies of Jafarnejad et al. DICER expression was found to be reduced in melanoma. Here, reduction of expression correlated with melanoma progression. They further revealed that DICER expression is controlled by the transcriptional regulator Sox4. Knockdown of Sox4 resulted in changes in miR expression pattern which could be demonstrated to be mainly dependent on DICER regulation (Jafarnejad et al., 2013). Two other studies, by contrast, revealed upregulation of DICER in melanoma samples (Ma et al., 2011; Sand et al., 2011). Interestingly, Sand et al. only determined differences in DICER expression on protein but not on mRNA level (Sand et al., 2012; Sand et al., 2013). This discrepancy between mRNA and protein level is also true for expression of AGO2 in melanoma. Here, Völler et al., detected strong downregulation of AGO2 on protein level in melanoma which was not observed on mRNA level by Sand et al. (Sand et al., 2012; Voller et al., 2013b). On protein level, further reduction of DROSHA expression was observed during melanoma progression by Jafarnejad et al. (Jafarnejad et al., 2013). Here, specificity of DROSHA detection was confirmed using two independent antibodies.

Further miRNA processing enzyme levels were only addressed at the mRNA level (Sand et al., 2012). Reduced expression of AGO1, TARBP2 and SND1 mRNA in primary melanoma compared to benign nevi was determined which needs to be confirmed at the protein level.

Until today, no studies on mutations of miRNA processing enzymes in melanoma are published, however, these were described in several other kinds of cancer. Recurrent mutations in DROSHA were observed in 12% of Wilms tumor samples, with E1147K found in 81% of these. Further, non-recurrent mutations in DGCR8, DICER1, XPO5 and TARBP2 were identified. Interestingly, this led to downregulation of a defined set of miRNAs (Torrezan et al., 2014).

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Analyzing the COSMIC database (table 1), mutation in proteins of the miR processing machinery can only rarely be found in melanoma with no obvious recurrent mutations or hot spots. Interestingly, several mutations are affecting prolines, which suggest an impact of the mutation on the structure of the respective protein. Further, mutations situated in PIWI or PAZ domains are observed potentially affecting their functions. In AGO1,3 and 4 four mutations are located in the far C-Terminal part (Q838*, D852N, T853I, R839W of the total protein length of 861/862). This is of interest as no specific domain was found here. In total, 26 mutations were observed revealing mutations in 8.2% of samples analyzed. However, 5 patients harbor more than one mutation or mutations in more than one protein.

In general, strong deregulation of proteins involved in miRNA processing can be found regularly in melanoma. Until today, the mechanistic impact of these changes is not clear. In the individual studies, the potential effects were addressed by targeting the respective protein. However, as several of the pathway members are regulated the whole system is complex and needs to be addressed in a more general fashion.

Deregulation of long noncoding RNAs was observed in several kinds of cancer including melanoma (Khaitan et al., 2011; Li et al., 2014b; Tang et al., 2013), see section on miR in melanoma. A new study in colon cancer revealed that re-expression of the lncRNA Uc.283+A in cancer cell lines leads to deregulation of a defined set of miRNA. Here, expression of miR-195 was most strongly modulated (Liz et al., 2014). Interestingly, the group could define the molecular mechanism. The lncRNA Uc.283+A blocks interaction of DGCR8 with the pri-MiR-195 by binding to the miR in a complementary region. This region is situated immediately upstream of the DROSHA/DGCR8 cleavage site modulating miR processing. This mechanism of regulation is new and different to ceRNA, which sequester miRNAs (Salmena et al., 2011). LncRNAs have been linked to melanoma development in several studies as illustrated before, however, no direct link was drawn to miRNA processing until today. A diagram, illustrating novel data on the involvement of miRs and members of microRNA processing machinery in melanocyte development and melanoma is shown in figure 1.

Finally, microRNA research in animal model of melanoma promises to yield important information on how differential expression of microRNAs (and the circuitries that they

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regulate) have been established over time. Analysis of models with specific genetic lesions, engineered melanoma-initiating cells and controlled environment can be compared with snap-shots of human melanoma individual cases and uncover the complete microRNA evolutive history of the tumor with obvious benefits for strategic treatments.

6 - Global networks controlled by micro-RNAs

A complex network of interactions between microRNAs, target genes, transcription factors and proteins involved in molecular processing (RBP, RNAses, Polymerases and component of the splicing machinery) is emerging from these studies, increasing enormously the complexity levels compared to classical pathway analysis. Functional analysis of microRNAs relies strongly on correlative levels of both microRNAs and their targets and so far the vast majority of studies, which addressed this point, were conducted on one miR-one gene interaction at the time. However correlative deep sequencing data of global microRNA/global mRNA expression in the same samples is now being collected for cancer samples by the TGCA (http://cancergenome.nih.gov/) and ICGC (https://icgc.org/), and starts to include proteome levels and dynamic time point analysis. One of the big obstacles is the problem of integrating heterogeneous data sets, derived from different platforms (i.e. microarray vs. deep sequencing). Bioinformatic approaches aimed at identifying the rules of interactions and system biology approaches catching the complex relationships and frequent regulatory loops where microRNAs are involved, start to be developed. Indeed, many computational methods aimed at identifying microRNA-target interactions have proved to be effective tools in assisting with the design of wet experiments, short-listing statistically significant regulatory interactions, thus making it feasible to conduct validation experiments.

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Li (Li et al., 2014d) developed TargetScore to integrate miRNA-overexpression data and target information based on sequence data. They analysed 113 miRNA transfection experiment from Gene Expression Omnibus (GEO) data sets. The predictions were validated using mirTarBase (http://mirtarbase.mbc.nctu.edu.tw/), proteomic data in Baek et al. (Baek et al., 2008) and gene functional enrichment analysis. The transfection data and source codes are available at:
http:// www.bioconductor.org/packages/devel/bioc/html/TargetScore.html.
Jacobsen et al. (Jacobsen et al., 2013) analyzed miRNA–target interactions across

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diverse cancer types. To explore the recurrence of these associations across cancer types, the authors developed a statistical score (REC - recurrence score). The top-ranked miRNA–mRNA interactions are those that have strong association across different cancer types. They applied the method to 11 different cancer types in The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih. gov/tcga/tcgaHome2.jsp) and reported all the results as an online resource at http://cancerminer.org.

Muniategui et al. (Muniategui A, 2013) analysed microRNA/mRNA data to identify miRNA–mRNA regulatory relationships, miRNA–mRNA regulatory modules and uncover miRNA–TF co-regulatory relationships. In order to discover miRNA-mRNA modules, Zhang et al. (Zhang et al., 2014b) proposed to integrate miRNA target predictions based on sequence data, miRNA and gene expression profiles, protein–protein interaction and DNA–protein interaction networks. miRNA target predictions were considered as static data, prior network, and expression profiles were used to identify the active miRNA–gene interactions. These active interactions were further refined by gene–gene interaction networks (protein–protein and DNA–protein networks).

Le et al. (Le TD, 2014) developed a regression-based method to integrate sequence, expression and protein interactions data for identifying modules of miRNAs and mRNAs for a specific condition. The method was applied to multiple cancer data sets from TCGA to identify the regulators (miRNAs) that are common for all cancer types and specific active regulators for each cancer type. The results were then validated against literature knowledge and by gene functional enrichment analysis.

Co-regulatory relationship between miRNAs and transcription factor (TF) are inferred by the existence of mRNAs that are targets of both regulators. To discover miRNA and transcription factor converging on similar targets, gene regulatory networks with both TFs and miRNAs based on sequence data were generated. A common framework of exploring miRNA–TF co-regulatory relationships is to integrate the putative target information of both TFs and miRNAs to obtain an interaction network with the three components, miRNAs, TFs and mRNAs. The combined network was then analyzed to identify the shared targets. It was found that the hubs of interactions are usually the TFs and network motifs that involve miRNAs, TFs and mRNAs were discovered (Chen et al., 2011; Shalgi et al., 2007; Tran and Mochida, 2010).

To create a tool allowing wet scientists to generate hypotheses and for experimental planning, Huang et al. (Huang et al., 2011) developed a web tool (mirConnX) for

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constructing regulatory networks that include miRNAs, TFs and mRNAs. Using this tool, users can input the expression data and receive as output a regulatory network that includes miRNA, TFs and mRNAs. However, these networks simply show statistical association in expression levels between regulators and target genes, serving as a framework for hypothesis validation. Thus, approaches based on sequence data provide a list of potential target genes. However, the results from these approaches involve a high rate of false discoveries. There is a need to design new accurate methods for using sequence data and combine them with data from "wet" labs. Indeed, experimental methods such as HITS-CLIP (Chi et al., 2009) and PAR-CLIP (Hafner et al., 2010) achieve better accuracy rates compared with prior prediction programs.

Complementary to sequence-based approaches, which discover static miRNA targets, approaches that use gene expression data can infer the miRNA activities and miRNA–mRNA relationships in a specific condition. However, these associative methods do not prove causality. Recently, causality discovery-based methods have emerged and provided an alternative approach to inferring miRNA–mRNA causal regulatory relationships. Therefore, it is crucial to design a new class of methods that can infer causal relationships as well as produce stable results across different data sets (Zhang et al., 2014b). Clearly, more experimental data are necessary.

Finally, a network based on skin and melanocyte like the ones constructed in 12 different tissues by Guo et al. (Guo et al., 2014b) would increase our predictions and validation in this field.

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7 - Conclusions

Research on miRNAs strongly evolved in cancer research. Many studies, some detailed in this review, addressed specific miRNAs of importance for melanoma. Further, researcher started to analyze miRNA processing and the processing machinery and try to understand the involvement in melanoma development and progression. Due to the complexity of the field, an overview and a more general understanding is still missing and the field is hindered by a very focused view on certain aspects/molecules. However, pathways and links are constantly developed. In addition, regularly functional assay are performed as final read out. Of course an impact of each miRNAs or the respective target gene on cell proliferation and migration is of crucial importance and has to be addressed in the individual studies.

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Finally, we need to understand the global network of “melano-miRs” including all regulated pathways and the molecular interaction. We further have to keep in mind that transcriptional control is the base for all regulations. Here, more studies linking mRNA expression, miRNA expression, protein expression and cellular effects are needed in the future.

We all agree that miRNA are potential therapeutic and diagnostic tools which can become very useful in the future. We have to keep in mind that the detailed understanding of this complex network is of crucial importance for successful therapeutic attempts. Our knowledge on protein and protein function, e.g. BRAF, developed and increased over decades, still many pitfalls and “surprises” came up while targeting these molecules. miRNAs are at least as complicated, targeting several genes with still unknown specificity and target choice. Here, more research input is needed.

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For Peer Review

References

*Abdel-Malek, Z. A., Knittel, J., Kadekaro, A. L., Swope, V. B., and Starner, R. (2008). The melanocortin 1 receptor and the UV response of human melanocytes--a shift in paradigm. Photochem Photobiol 84***,** *501-8.*

*Adijanto, J., Castorino, J. J., Wang, Z. X., Maminishkis, A., Grunwald, G. B., and Philp, N. J. (2012). Microphthalmia-associated transcription factor (MITF) promotes differentiation of human retinal pigment epithelium (RPE) by regulating microRNAs-204/211 expression. J Biol Chem 287***,** *20491-503.*

*Amaral, P. P., and Mattick, J. S. (2008). Noncoding RNA in development. Mamm Genome 19***,** *454-92.*

*Baek, D., Villen, J., Shin, C., Camargo, F. D., Gygi, S. P., and Bartel, D. P. (2008). The impact of microRNAs on protein output. Nature 455***,** *64-71.*

*Baxter, L. L., Loftus, S. K., and Pavan, W. J. (2009). Networks and pathways in pigmentation, health, and disease. Wiley Interdiscip Rev Syst Biol Med 1***,** *359-71.*

*Bemis, L. T., Chen, R., Amato, C. M., Classen, E. H., Robinson, S. E., Coffey, D. G., Erickson, P. F., Shellman, Y. G., and Robinson, W. A. (2008). MicroRNA-137 targets microphthalmia-associated transcription factor in melanoma cell lines. Cancer Res 68***,** *1362-8.*

*Berezikov, E., Chung, W. J., Willis, J., Cuppen, E., and Lai, E. C. (2007). Mammalian mirtron genes. Mol Cell 28***,** *328-36.*

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Bizuayehu, T. T., Lanes, C. F., Furmanek, T., Karlsen, B. O., Fernandes, J. M., Johansen, S. D., and Babiak, I. (2012). Differential expression patterns of conserved miRNAs and isomiRs during Atlantic halibut development. BMC Genomics 13, *11.*

Botchkareva, N. V. (2012). MicroRNA/mRNA regulatory networks in the control of skin development and regeneration. Cell Cycle 11, *468-74.*

Bu, P., and Yang, P. (2014). MicroRNA-203 inhibits malignant melanoma cell migration by targeting versican. Exp Ther Med 8, *309-315.*

Caramuta, S., Egyhazi, S., Rodolfo, M., Witten, D., Hansson, J., Larsson, C., and Lui, W. O. (2010). MicroRNA expression profiles associated with mutational status and survival in malignant melanoma. J Invest Dermatol 130, *2062-70.*

Carreira, S., Goodall, J., Aksan, I., La Rocca, S. A., Galibert, M. D., Denat, L., Larue, L., and Goding, C. R. (2005). Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature 433, *764-9.*

Chakravarti, D., Su, X., Cho, M. S., Bui, N. H., Coarfa, C., Venkatanarayan, A., Benham, A. L., Flores Gonzalez, R. E., Alana, J., Xiao, W., et al. (2014). Induced multipotency in adult keratinocytes through down-regulation of DeltaNp63 or DGCR8. Proc Natl Acad Sci U S A 111, *E572-81.*

Chan, E., Patel, R., Nallur, S., Ratner, E., Bacchiocchi, A., Hoyt, K., Szpakowski, S., Godshalk, S., Ariyan, S., Sznol, M., et al. (2011). MicroRNA signatures differentiate melanoma subtypes. Cell Cycle 10, *1845-52.*

Chen, C. Y., Chen, S. T., Fuh, C. S., Juan, H. F., and Huang, H. C. (2011). Coregulation of transcription factors and microRNAs in human transcriptional regulatory network. BMC Bioinformatics 12 Suppl 1, *S41.*

Chen, J., Feilotter, H. E., Pare, G. C., Zhang, X., Pemberton, J. G., Garady, C., Lai, D., Yang, X., and Tron, V. A. (2010). MicroRNA-193b represses cell proliferation and regulates cyclin D1 in melanoma. Am J Pathol 176, *2520-9.*

Chendrimada, T. P., Gregory, R. I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K., and Shiekhattar, R. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436, *740-4.*

Chi, S. W., Zang, J. B., Mele, A., and Darnell, R. B. (2009). Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. Nature 460, *479-86.*

Chiang, H. R., Schoenfeld, L. W., Ruby, J. G., Auyeung, V. C., Spies, N., Baek, D., Johnston, W. K., Russ, C., Luo, S., Babiarz, J. E., et al. (2010). Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. Genes Dev 24, *992-1009.*

Cordes, K. R., and Srivastava, D. (2009). MicroRNA regulation of cardiovascular development. Circ Res 104, *724-32.*

Couts, K. L., Anderson, E. M., Gross, M. M., Sullivan, K., and Ahn, N. G. (2013). Oncogenic B-Raf signaling in melanoma cells controls a network of microRNAs with combinatorial functions. Oncogene 32, *1959-70.*

D'adda Di Fagagna, F. (2014). A direct role for small non-coding RNAs in DNA damage response. Trends Cell Biol 24, *171-8.*

Diederichs, S., and Haber, D. A. (2006). Sequence variations of microRNAs in human cancer: alterations in predicted secondary structure do not affect processing. Cancer Res 66, *6097-104.*

Dong, C., Wang, H., Xue, L., Dong, Y., Yang, L., Fan, R., Yu, X., Tian, X., Ma, S., and Smith, G. W. (2012). Coat color determination by miR-137 mediated down-regulation of microphthalmia-associated transcription factor in a mouse model. Rna 18, *1679-86.*

Dynoodt, P., Mestdagh, P., Van Peer, G., Vandesompele, J., Goossens, K., Peelman, L. J., Geusens, B., Speeckaert, R. M., Lambert, J. L., and Van Gele, M. J. (2013). Identification of miR-145 as a key regulator of the pigmentary process. J Invest Dermatol 133, *201-9.*

Eberhart, J. K., He, X., Swartz, M. E., Yan, Y. L., Song, H., Boling, T. C., Kunerth, A. K., Walker, M. B., Kimmel, C. B., and Postlethwait, J. H. (2008). MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis. Nat Genet 40, *290-8.*

For Peer Review

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51
52
53
54
55
56
57
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Eller, M. S., Maeda, T., Magnoni, C., Atwal, D., and Gilchrest, B. A. (1997). Enhancement of DNA repair in human skin cells by thymidine dinucleotides: evidence for a p53-mediated mammalian SOS response. Proc Natl Acad Sci U S A 94, *12627-32.*

Fang, Z., and Rajewsky, N. (2011). The impact of miRNA target sites in coding sequences and in 3'UTRs. PLoS One 6, *e18067.*

Francia, S., Michelini, F., Saxena, A., Tang, D., De Hoon, M., Anelli, V., Mione, M., Carninci, P., and D'adda Di Fagagna, F. (2012). Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 488, *231-5.*

Garcia-Lopez, J., Hourcade Jde, D., and Del Mazo, J. (2013). Reprogramming of microRNAs by adenosine-to-inosine editing and the selective elimination of edited microRNA precursors in mouse oocytes and preimplantation embryos. Nucleic Acids Res 41, *5483-93.*

Georgantas, R. W., 3rd, Streicher, K., Luo, X., Greenlees, L., Zhu, W., Liu, Z., Brohawn, P., Morehouse, C., Higgs, B. W., Richman, L., et al. (2014). MicroRNA-206 induces G1 arrest in melanoma by inhibition of CDK4 and Cyclin D. Pigment Cell Melanoma Res 27, *275-86.*

Goding, C. R. (2000). Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. Genes Dev 14, *1712-28.*

Godshalk, S. E., Paranjape, T., Nallur, S., Speed, W., Chan, E., Molinaro, A. M., Bacchiocchi, A., Hoyt, K., Tworkoski, K., Stern, D. F., et al. (2011). A Variant in a MicroRNA complementary site in the 3' UTR of the KIT oncogene increases risk of acral melanoma. Oncogene 30, *1542-50.*

Goodarzi, H., Zhang, S., Buss, C. G., Fish, L., Tavazoie, S., and Tavazoie, S. F. (2014). Metastasis-suppressor transcript destabilization through TARBP2 binding of mRNA hairpins. Nature 513, *256-60.*

Goswami, S., Tarapore, R. S., Teslaa, J. J., Grinblat, Y., Setaluri, V., and Spiegelman, V. S. (2010). MicroRNA-340-mediated degradation of microphthalmia-associated transcription factor mRNA is inhibited by the coding region determinant-binding protein. J Biol Chem 285, *20532-40.*

Greenberg, E., Hajdu, S., Nemlich, Y., Cohen, R., Itzhaki, O., Jacob-Hirsch, J., Besser, M. J., Schachter, J., and Markel, G. (2014). Differential regulation of aggressive features in melanoma cells by members of the miR-17-92 complex. Open Biol 4, *140030.*

Griffiths-Jones, S., Saini, H. K., Van Dongen, S., and Enright, A. J. (2008). miRBase: tools for microRNA genomics. Nucleic Acids Res 36, *D154-8.*

Guo, F., Carter, D. E., and Leask, A. (2014a). miR-218 regulates focal adhesion kinase-dependent TGFbeta signaling in fibroblasts. Mol Biol Cell 25, *1151-8.*

Guo, Z., Maki, M., Ding, R., Yang, Y., Zhang, B., and Xiong, L. (2014b). Genome-wide survey of tissue-specific microRNA and transcription factor regulatory networks in 12 tissues. Sci Rep 4, *5150.*

Hafner, M., Landthaler, M., Burger, L., Khorshid, M., Hausser, J., Berninger, P., Rothballer, A., Ascano, M., Jungkamp, A. C., Munschauer, M., et al. (2010). PAR-CliP--a method to identify transcriptome-wide the binding sites of RNA binding proteins. J Vis Exp.

Hansen, T. B., Jensen, T. I., Clausen, B. H., Bramsen, J. B., Finsen, B., Damgaard, C. K., and Kjems, J. (2013). Natural RNA circles function as efficient microRNA sponges. Nature 495, *384-8.*

Helwak, A., Kudla, G., Dudnakova, T., and Tollervey, D. (2013). Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. Cell 153, *654-65.*

Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A., and Arnheiter, H. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell 74, *395-404.*

Hoek, K. S., and Goding, C. R. (2010). Cancer stem cells versus phenotype-switching in melanoma. Pigment Cell Melanoma Res 23, *746-59.*

Hofreiter, M., and Schoneberg, T. (2010). The genetic and evolutionary basis of colour variation in vertebrates. Cell Mol Life Sci 67, *2591-603.*

Hou, L., Arnheiter, H., and Pavan, W. J. (2006). Interspecies difference in the regulation of melanocyte development by SOX10 and MITF. Proc Natl Acad Sci U S A 103, *9081-5.*

Huang, G. T., Athanassiou, C., and Benos, P. V. (2011). mirConnX: condition-specific mRNA-microRNA network integrator. Nucleic Acids Res 39, *W416-23.*

For Peer Review

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Huang, H., Xie, C., Sun, X., Ritchie, R. P., Zhang, J., and Chen, Y. E. (2010a). miR-10a contributes to retinoid acid-induced smooth muscle cell differentiation. J Biol Chem 285, 9383-9.

Huang, T., Liu, Y., Huang, M., Zhao, X., and Cheng, L. (2010b). Wnt1-cre-mediated conditional loss of Dicer results in malformation of the midbrain and cerebellum and failure of neural crest and dopaminergic differentiation in mice. J Mol Cell Biol 2, 152-63.

Humphreys, D. T., Hynes, C. J., Patel, H. R., Wei, G. H., Cannon, L., Fatkin, D., Suter, C. M., Clancy, J. L., and Preiss, T. (2012). Complexity of murine cardiomyocyte miRNA biogenesis, sequence variant expression and function. PLoS One 7, e30933.

Hwang, H. W., Baxter, L. L., Loftus, S. K., Cronin, J. C., Trivedi, N. S., Borate, B., and Pavan, W. J. (2014). Distinct microRNA expression signatures are associated with melanoma subtypes and are regulated by HIF1A. Pigment Cell Melanoma Res 27, 777-87.

Ivey, K. N., and Srivastava, D. (2010). MicroRNAs as regulators of differentiation and cell fate decisions. Cell Stem Cell 7, 36-41.

Jacobsen, A., Silber, J., Harinath, G., Huse, J. T., Schultz, N., and Sander, C. (2013). Analysis of microRNA-target interactions across diverse cancer types. Nat Struct Mol Biol 20, 1325-32.

Jafarnejad, S. M., Ardekani, G. S., Ghaffari, M., Martinka, M., and Li, G. (2013). Sox4-mediated Dicer expression is critical for suppression of melanoma cell invasion. Oncogene 32, 2131-9.

Jian, Q., An, Q., Zhu, D., Hui, K., Liu, Y., Chi, S., and Li, C. (2014). MicroRNA 340 Is Involved in UVB-Induced Dendrite Formation through the Regulation of RhoA Expression in Melanocytes. Mol Cell Biol 34, 3407-20.

Kappelmann, M., Kuphal, S., Meister, G., Vardimon, L., and Bosserhoff, A. K. (2013). MicroRNA miR-125b controls melanoma progression by direct regulation of c-Jun protein expression. Oncogene 32, 2984-91.

Karreth, F. A., Tay, Y., Perna, D., Ala, U., Tan, S. M., Rust, A. G., Denicola, G., Webster, K. A., Weiss, D., Perez-Mancera, P. A., et al. (2011). In vivo identification of tumor- suppressive PTEN ceRNAs in an oncogenic BRAF-induced mouse model of melanoma. Cell 147, 382-95.

Katoh, T., Sakaguchi, Y., Miyauchi, K., Suzuki, T., Kashiwabara, S., and Baba, T. (2009). Selective stabilization of mammalian microRNAs by 3' adenylation mediated by the cytoplasmic poly(A) polymerase GLD-2. Genes Dev 23, 433-8.

Kawahara, Y., Zinshteyn, B., Chendrimada, T. P., Shiekhattar, R., and Nishikura, K. (2007a). RNA editing of the microRNA-151 precursor blocks cleavage by the Dicer-TRBP complex. EMBO Rep 8, 763-9.

Kawahara, Y., Zinshteyn, B., Sethupathy, P., Iizasa, H., Hatzigeorgiou, A. G., and Nishikura, K. (2007b). Redirection of silencing targets by adenosine-to-inosine editing of miRNAs. Science 315, 1137-40.

Kelsh, R. N. (2004). Genetics and evolution of pigment patterns in fish. Pigment Cell Res 17, 326-36.

Kennell, J. A., Cadigan, K. M., Shakhmantsir, I., and Waldron, E. J. (2012). The microRNA miR-8 is a positive regulator of pigmentation and eclosion in Drosophila. Dev Dyn 241, 161-8.

Khaitan, D., Dinger, M. E., Mazar, J., Crawford, J., Smith, M. A., Mattick, J. S., and Perera, R. J. (2011). The melanoma-upregulated long noncoding RNA SPRY4-IT1 modulates apoptosis and invasion. Cancer Res 71, 3852-62.

Kim, K. H., Bin, B. H., Kim, J., Dong, S. E., Park, P. J., Choi, H., Kim, B. J., Yu, S. J., Kang, H., Kang, H. H., et al. (2014). Novel inhibitory function of miR-125b in melanogenesis. Pigment Cell Melanoma Res 27, 140-4.

Kozubek, J., Ma, Z., Fleming, E., Duggan, T., Wu, R., Shin, D. G., and Dadras, S. S. (2013). In-depth characterization of microRNA transcriptome in melanoma. PLoS One 8, e72699.

Krol, J., Loedige, I., and Filipowicz, W. (2010). The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11, 597-610.

Lang, D., Lu, M. M., Huang, L., Engleka, K. A., Zhang, M., Chu, E. Y., Lipner, S., Skoultchi, A., Millar, S. E., and Epstein, J. A. (2005). Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature 433, 884-7.

Le Td, L. L., Zhang J, Liu B, Li J (2014). From miRNA regulation to miRNA-TF co-regulation: computational approaches and challenges. Brief Bioinform in press.

For Peer Review

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Lee, R. C., Feinbaum, R. L., and Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843-54.

Leibowitz-Amit, R., Sidi, Y., and Avni, D. (2012). Aberrations in the micro-RNA biogenesis machinery and the emerging roles of micro-RNAs in the pathogenesis of cutaneous malignant melanoma. Pigment Cell Melanoma Res 25, 740-57.

Leucci, E., Patella, F., Waage, J., Holmstrom, K., Lindow, M., Porse, B., Kauppinen, S., and Lund, A. H. (2013). microRNA-9 targets the long non-coding RNA MALAT1 for degradation in the nucleus. Sci Rep 3, 2535.

Levati, L., Alvino, E., Pagani, E., Arcelli, D., Caporaso, P., Bondanza, S., Di Leva, G., Ferracin, M., Volinia, S., Bonmassar, E., et al. (2009). Altered expression of selected microRNAs in melanoma: antiproliferative and proapoptotic activity of miRNA-155. Int J Oncol 35, 393-400.

Levy, C., Khaled, M., Robinson, K. C., Veguilla, R. A., Chen, P. H., Yokoyama, S., Makino, E., Lu, J., Larue, L., Beermann, F., et al. (2010). Lineage-specific transcriptional regulation of DICER by MITF in melanocytes. Cell 141, 994-1005.

Li, L., Wang, D., Xue, M., Mi, X., Liang, Y., and Wang, P. (2014a). 3'UTR shortening identifies high-risk cancers with targeted dysregulation of the ceRNA network. Sci Rep 4, 5406.

Li, R., Zhang, L., Jia, L., Duan, Y., Li, Y., Bao, L., and Sha, N. (2014b). Long non-coding RNA BANCR promotes proliferation in malignant melanoma by regulating MAPK pathway activation. PLoS One 9, e100893.

Li, R., Zhang, L., Jia, L., Duan, Y., Li, Y., Wang, J., Bao, L., and Sha, N. (2014c). MicroRNA-143 targets Syndecan-1 to repress cell growth in melanoma. PLoS One 9, e94855.

Li, S. C., Liao, Y. L., Chan, W. C., Ho, M. R., Tsai, K. W., Hu, L. Y., Lai, C. H., Hsu, C. N., and Lin, W. C. (2011). Interrogation of rabbit miRNAs and their isomiRs. Genomics 98, 453-9.

Li, Y., Goldenberg, A., Wong, K. C., and Zhang, Z. (2014d). A probabilistic approach to explore human miRNA targetome by integrating miRNA-overexpression data and sequence information. Bioinformatics 30, 621-8.

Liz, J., Portela, A., Soler, M., Gomez, A., Ling, H., Michlewski, G., Calin, G. A., Guil, S., and Esteller, M. (2014). Regulation of pri-miRNA processing by a long noncoding RNA transcribed from an ultraconserved region. Mol Cell 55, 138-47.

Luciano, D. J., Mirsky, H., Vendetti, N. J., and Maas, S. (2004). RNA editing of a miRNA precursor. Rna 10, 1174-7.

Luo, C., Weber, C. E., Osen, W., Bosserhoff, A. K., and Eichmuller, S. B. (2014). The role of microRNAs in melanoma. Eur J Cell Biol 93, 11-22.

Ma, Z., Swede, H., Cassarino, D., Fleming, E., Fire, A., and Dadras, S. S. (2011). Up-regulated Dicer expression in patients with cutaneous melanoma. PLoS One 6, e20494.

Mcgill, G. G., Horstmann, M., Widlund, H. R., Du, J., Motyckova, G., Nishimura, E. K., Lin, Y. L., Ramaswamy, S., Avery, W., Ding, H. F., et al. (2002). Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell 109, 707-18.

Melo, S. A., Moutinho, C., Ropero, S., Calin, G. A., Rossi, S., Spizzo, R., Fernandez, A. F., Davalos, V., Villanueva, A., Montoya, G., et al. (2010). A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. Cancer Cell 18, 303-15.

Mills, M. G., and Patterson, L. B. (2009). Not just black and white: pigment pattern development and evolution in vertebrates. Semin Cell Dev Biol 20, 72-81.

Miyamura, Y., Coelho, S. G., Wolber, R., Miller, S. A., Wakamatsu, K., Zmudzka, B. Z., Ito, S., Smuda, C., Passeron, T., Choi, W., et al. (2007). Regulation of human skin pigmentation and responses to ultraviolet radiation. Pigment Cell Res 20, 2-13.

Mollaaghababa, R., and Pavan, W. J. (2003). The importance of having your SOX on: role of SOX10 in the development of neural crest-derived melanocytes and glia. Oncogene 22, 3024-34.

Mori, M., Triboulet, R., Mohseni, M., Schlegelmilch, K., Shrestha, K., Camargo, F. D., and Gregory, R. I. (2014). Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. Cell 156, 893-906.

For Peer Review

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11
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15
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Morin, R. D., O'connor, M. D., Griffith, M., Kuchenbauer, F., Delaney, A., Prabhu, A. L., Zhao, Y., Mcdonald, H., Zeng, T., Hirst, M., et al. (2008). Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells. Genome Res 18, 610-21.

Mueller, D. W., and Bosserhoff, A. K. (2010). The evolving concept of 'melano-miRs'-microRNAs in melanomagenesis. Pigment Cell Melanoma Res 23, 620-6.

Muniategui A, P. J., Planes Fj, Rubio A. (2013). Joint analysis of miRNA and mRNA expression data. Brief Bioinform 14, 263-78.

Nemlich, Y., Greenberg, E., Ortenberg, R., Besser, M. J., Barshack, I., Jacob-Hirsch, J., Jacoby, E., Eyal, E., Rivkin, L., Prieto, V. G., et al. (2013). MicroRNA-mediated loss of ADAR1 in metastatic melanoma promotes tumor growth. J Clin Invest 123, 2703-18.

Nie, X., Wang, Q., and Jiao, K. (2011). Dicer activity in neural crest cells is essential for craniofacial organogenesis and pharyngeal arch artery morphogenesis. Mech Dev 128, 200-7.

Noguchi, S., Mori, T., Otsuka, Y., Yamada, N., Yasui, Y., Iwasaki, J., Kumazaki, M., Maruo, K., and Akao, Y. (2012). Anti-oncogenic microRNA-203 induces senescence by targeting E2F3 protein in human melanoma cells. J Biol Chem 287, 11769-77.

Nyholm, A. M., Lerche, C. M., Manfe, V., Biskup, E., Johansen, P., Morling, N., Thomsen, B. M., Glud, M., and Gniadecki, R. (2014). miR-125b induces cellular senescence in malignant melanoma. BMC Dermatol 14, 8.

Ohbayashi, N., and Fukuda, M. (2012). Role of Rab family GTPases and their effectors in melanosomal logistics. J Biochem 151, 343-51.

Orom, U. A., Nielsen, F. C., and Lund, A. H. (2008). MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 30, 460-71.

Osawa, M., Egawa, G., Mak, S. S., Moriyama, M., Freter, R., Yonetani, S., Beermann, F., and Nishikawa, S. (2005). Molecular characterization of melanocyte stem cells in their niche. Development 132, 5589-99.

Palanichamy, J. K., and Rao, D. S. (2014). miRNA dysregulation in cancer: towards a mechanistic understanding. Front Genet 5, 54.

Pasmant, E., Laurendeau, I., Heron, D., Vidaud, M., Vidaud, D., and Bieche, I. (2007). Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. Cancer Res 67, 3963-9.

Pasquinelli, A. E., and Ruvkun, G. (2002). Control of developmental timing by micrornas and their targets. Annu Rev Cell Dev Biol 18, 495-513.

Pedace, L., Cozzolino, A. M., Barboni, L., De Bernardo, C., Grammatico, P., De Simone, P., Buccini, P., Ferrari, A., Catricala, C., Colombo, T., et al. (2014). A novel variant in the 3' untranslated region of the CDK4 gene: interference with microRNA target sites and role in increased risk of cutaneous melanoma. Cancer Genet 207, 168-9.

Philippidou, D., Schmitt, M., Moser, D., Margue, C., Nazarov, P. V., Muller, A., Vallar, L., Nashan, D., Behrmann, I., and Kreis, S. (2010). Signatures of microRNAs and selected microRNA target genes in human melanoma. Cancer Res 70, 4163-73.

Poenitzsch Strong, A. M., Setaluri, V., and Spiegelman, V. S. (2014). microRNA-340 as a modulator of RAS-RAF-MAPK signaling in melanoma. Arch Biochem Biophys.

Poliseno, L., Haimovic, A., Segura, M. F., Hanniford, D., Christos, P. J., Darvishian, F., Wang, J., Shapiro, R. L., Pavlick, A. C., Berman, R. S., et al. (2012). Histology-specific microRNA alterations in melanoma. J Invest Dermatol 132, 1860-8.

Ramalingam, P., Palanichamy, J. K., Singh, A., Das, P., Bhagat, M., Kassab, M. A., Sinha, S., and Chattopadhyay, P. (2014). Biogenesis of intronic miRNAs located in clusters by independent transcription and alternative splicing. Rna 20, 76-87.

Saleiban, A., Faxalv, L., Claesson, K., Jonsson, J. I., and Osman, A. (2014). miR-20b regulates expression of proteinase-activated receptor-1 (PAR-1) thrombin receptor in melanoma cells. Pigment Cell Melanoma Res 27, 431-41.

Salmena, L., Poliseno, L., Tay, Y., Kats, L., and Pandolfi, P. P. (2011). A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146, 353-8.

For Peer Review

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52
53
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Sand, M., Gambichler, T., Sand, D., Altmeyer, P., Stuecker, M., and Bechara, F. G. (2011). Immunohistochemical expression patterns of the microRNA-processing enzyme Dicer in cutaneous malignant melanomas, benign melanocytic nevi and dysplastic melanocytic nevi. Eur J Dermatol 21, 18-21.

Sand, M., Skrygan, M., Georgas, D., Sand, D., Gambichler, T., Altmeyer, P., and Bechara, F. G. (2012). The miRNA machinery in primary cutaneous malignant melanoma, cutaneous malignant melanoma metastases and benign melanocytic nevi. Cell Tissue Res 350, 119-26.

Sand, M., Skrygan, M., Sand, D., Georgas, D., Gambichler, T., Hahn, S. A., Altmeyer, P., and Bechara, F. G. (2013). Comparative microarray analysis of microRNA expression profiles in primary cutaneous malignant melanoma, cutaneous malignant melanoma metastases, and benign melanocytic nevi. Cell Tissue Res 351, 85-98.

Sato-Jin, K., Nishimura, E. K., Akasaka, E., Huber, W., Nakano, H., Miller, A., Du, J., Wu, M., Hanada, K., Sawamura, D., et al. (2008). Epistatic connections between microphthalmia-associated transcription factor and endothelin signaling in Waardenburg syndrome and other pigmentary disorders. Faseb J 22, 1155-68.

Segura, M. F., Belitskaya-Levy, I., Rose, A. E., Zakrzewski, J., Gaziel, A., Hanniford, D., Darvishian, F., Berman, R. S., Shapiro, R. L., Pavlick, A. C., et al. (2010). Melanoma MicroRNA signature predicts post-recurrence survival. Clin Cancer Res 16, 1577-86.

Shalgi, R., Lieber, D., Oren, M., and Pilpel, Y. (2007). Global and local architecture of the mammalian microRNA-transcription factor regulatory network. PLoS Comput Biol 3, e131.

Stark, M. S., Tyagi, S., Nancarrow, D. J., Boyle, G. M., Cook, A. L., Whiteman, D. C., Parsons, P. G., Schmidt, C., Sturm, R. A., and Hayward, N. K. (2010). Characterization of the Melanoma miRNAome by Deep Sequencing. PLoS One 5, e9685.

Subramanyam, D., and Blelloch, R. (2011). From microRNAs to targets: pathway discovery in cell fate transitions. Curr Opin Genet Dev 21, 498-503.

Sun, V., Zhou, W. B., Majid, S., Kashani-Sabet, M., and Dar, A. A. (2014). MicroRNA-mediated regulation of melanoma. Br J Dermatol 171, 234-41.

Tang, L., Zhang, W., Su, B., and Yu, B. (2013). Long noncoding RNA HOTAIR is associated with motility, invasion, and metastatic potential of metastatic melanoma. Biomed Res Int 2013, 251098.

Tay, Y., Rinn, J., and Pandolfi, P. P. (2014). The multilayered complexity of ceRNA crosstalk and competition. Nature 505, 344-52.

Tay, Y., Zhang, J., Thomson, A. M., Lim, B., and Rigoutsos, I. (2008). MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455, 1124-8.

Tian, Y., Zhang, X., Hao, Y., Fang, Z., and He, Y. (2014). Potential roles of abnormally expressed long noncoding RNA UCA1 and Malat-1 in metastasis of melanoma. Melanoma Res 24, 335-41.

Torrezan, G. T., Ferreira, E. N., Nakahata, A. M., Barros, B. D., Castro, M. T., Correa, B. R., Krepischi, A. C., Olivieri, E. H., Cunha, I. W., Tabori, U., et al. (2014). Recurrent somatic mutation in DROSHA induces microRNA profile changes in Wilms tumour. Nat Commun 5, 4039.

Tran, L. S., and Mochida, K. (2010). A platform for functional prediction and comparative analyses of transcription factors of legumes and beyond. Plant Signal Behav 5, 550-2.

Voller, D., Ott, C., and Bosserhoff, A. (2013a). MicroRNAs in malignant melanoma. Clin Biochem 46, 909-17.

Voller, D., Reinders, J., Meister, G., and Bosserhoff, A. K. (2013b). Strong reduction of AGO2 expression in melanoma and cellular consequences. Br J Cancer 109, 3116-24.

Watanabe, A., Takeda, K., Ploplis, B., and Tachibana, M. (1998). Epistatic relationship between Waardenburg syndrome genes MITF and PAX3. Nat Genet 18, 283-6.

Wei, J., Shi, Y., Zheng, L., Zhou, B., Inose, H., Wang, J., Guo, X. E., Grosschedl, R., and Karsenty, G. (2012). miR-34s inhibit osteoblast proliferation and differentiation in the mouse by targeting SATB2. J Cell Biol 197, 509-21.

Wei, Y., Du, Y., Chen, X., Li, P., Wang, Y., Zang, W., Zhao, L., Li, Z., and Zhao, G. (2014). Expression patterns of microRNA-218 and its potential functions by targeting CIP2A and BMI1 genes in melanoma. Tumour Biol 35, 8007-15.

For Peer Review

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13
14
15
16
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51
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Wu, C. F., Tan, G. H., Ma, C. C., and Li, L. (2013). The non-coding RNA llme23 drives the malignant property of human melanoma cells. J Genet Genomics 40, *179-88.*

Wyman, S. K., Knouf, E. C., Parkin, R. K., Fritz, B. R., Lin, D. W., Dennis, L. M., Krouse, M. A., Webster, P. J., and Tewari, M. (2011). Post-transcriptional generation of miRNA variants by multiple nucleotidyl transferases contributes to miRNA transcriptome complexity. Genome Res 21, *1450-61.*

Xin, M., Small, E. M., Sutherland, L. B., Qi, X., Mcanally, J., Plato, C. F., Richardson, J. A., Bassel-Duby, R., and Olson, E. N. (2009). MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev 23, *2166-78.*

Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A., and Kosik, K. S. (2009). MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 137, *647-58.*

Xu, Y., Brenn, T., Brown, E. R., Doherty, V., and Melton, D. W. (2012). Differential expression of microRNAs during melanoma progression: miR-200c, miR-205 and miR-211 are downregulated in melanoma and act as tumour suppressors. Br J Cancer 106, *553-61.*

Yamaguchi, Y., Brenner, M., and Hearing, V. J. (2007). The regulation of skin pigmentation. J Biol Chem 282, *27557-61.*

Yan, B., Liu, B., Zhu, C. D., Li, K. L., Yue, L. J., Zhao, J. L., Gong, X. L., and Wang, C. H. (2013). microRNA regulation of skin pigmentation in fish. J Cell Sci 126, *3401-8.*

Yang, J., Li, T., Gao, C., Lv, X., Liu, K., Song, H., Xing, Y., and Xi, T. (2014). FOXO1 3'UTR functions as a ceRNA in repressing the metastases of breast cancer cells via regulating miRNA activity. FEBS Lett 588, *3218-24.*

Yang, J. S., Phillips, M. D., Betel, D., Mu, P., Ventura, A., Siepel, A. C., Chen, K. C., and Lai, E. C. (2011). Widespread regulatory activity of vertebrate microRNA species. Rna 17*, *312-26.*

Zehir, A., Hua, L. L., Maska, E. L., Morikawa, Y., and Cserjesi, P. (2010). Dicer is required for survival of differentiating neural crest cells. Dev Biol 340, *459-67.*

Zhang, J., Lu, L., Xiong, Y., Qin, W., Zhang, Y., Qian, Y., Jiang, H., and Liu, W. (2014a). MLK3 promotes melanoma proliferation and invasion and is a target of microRNA-125b. Clin Exp Dermatol 39, *376-84.*

Zhang, W., Zang, J., Jing, X., Sun, Z., Yan, W., Yang, D., Shen, B., and Guo, F. (2014b). Identification of candidate miRNA biomarkers from miRNA regulatory network with application to prostate cancer. J Transl Med 12, *66.*

Zhu Z, H. J., Jia X, Jiang J, Bai R, Yu X, Lv L, Fan R, He X, Geng J, You R, Dong Y, Qiao D, Lee Kb, Smith Gw, Dong C (2010). MicroRNA-25 functions in regulation of pigmentation by targeting the transcription factor MITF in Alpaca (Lama pacos) skin melanocytes. Domest Anim Endocrinol. 38, *200-209.*

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Figure Legend

Figure 1: Diagram illustrating the most recent findings (reported in the text) on microRNAs (red boxes), long ncRNAs (grey cloud) and miR processing enzymes (green boxes) in melanocyte development and melanoma. See text for detailed references. MicroRNAs involved in more than one stage/condition in melanocyte biology are similarly coloured. Black connecting lines indicate known or predicted (broken line) targets. N= notochord

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TABLE I

Analysis of mutations in miRNA processing proteins based on the COSMIC database. Tissue and cell lines were evaluated.

	mutations	aa changes/no of MM analysed	zygosity	Patient	function	subtype
Dicer1	P1432P P762S P750L E502K	3/319 (0.9%)	Het Het Het Het	24Z YUKLAB YUKLAB CP66-Mel	E502 in helicase C domain (502G in colonCA 1 sample)	
Drosha	P347L	1/319 (0.3%)	?	ME043T	?	
XPO5	R893H V1069I	2/319 (0.6%)	Het ?	MZ7-mel ME009T	?	
DGCR8	M1K P378L	2/314 (0.6%)	Het Het	Lau108 YUMER	ATG missing ?	
EIF2C1 (AGO1)	K49* N422N P321L E405E G420N G437G Q838*	4/314 (1.3%)	Het ? Het ? Het Het Het	Lau63 Me009t CP66-Mel cSCCP1 Lau63 Lau149 YUGAFFE	P321 in PAZ domain; Info: total length 858aa	
EIF2C2 (AGO2)	A627T P590S M549I Q486*	4/314 (1.3%)	Het Het Het Het	Lau618 YUZINO YURIF YUKIL	486: Stop before Piwi, 549,590,627 in Piwi	mucosal
EIF2C3 (AGO3)	S5V P97L R669W D852N T853I	5/314 (1.6%)	Het Het Het ? Het	YUDIALE YUWHIM YUKLAB ME032T YUZINO	total length 861; 853, 852 after Piwi, near C-term; 669 in Piwi	Mucosal mucosal
EIF2C4 (AGO4)	P9T P175S P614P Q654* G703R R839W	5/314 (1.6%)	Het Het Het ? Het Het	YUMER YURIDA Lau63 ME016T YUZINO YULAN	175 in DUF1785 domain; 614,654,703 in piwi; 839 after Piwi (total aa: 862)	Nodular mucosal
Total		8.2%				

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