miR-214 as a Key Hub that Controls Cancer Networks: Small Player, Multiple Functions

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MicroRNAs are short regulatory RNAs that are able to post-transcriptionally modulate gene expression and that have crucial roles in the control of physiological and pathological processes including cancer onset, growth, and progression. miR-214, located inside the sequence of the long noncoding *Dmn3os* transcript, contributes to the regulation of normal and cancer cell biology, even if it operates in a context-dependent and sometimes contradictory manner. miR-214 is deregulated in several human tumors including melanoma, breast, ovarian, gastric, and hepatocellular carcinomas. miR-214's pleiotropic and tumor-specific contribution to various cancer formation and progression hallmarks is achieved via its several target genes. In fact, miR-214 behaves as a key hub by coordinating fundamental signaling networks such as PTEN/AKT, β-catenin, and tyrosine kinase receptor pathways. Interestingly, miR-214 also regulates the levels of crucial gene expression modulators: the epigenetic repressor Ezh2, "genome guardian" p53, transcription factors TFAP2, and another microRNA, miR-148b. Thus, miR-214 seems to have essential roles in coordinating tumor proliferation, stemness, angiogenesis, invasiveness, extravasation, metastasis, resistance to chemotherapy, and microenvironment. The sum of current literature reports suggests that miR-214 is a molecular hub involved in the control of cancer networks and, as such, could be a potential diagnostic/prognostic biomarker and target for therapeutic intervention.

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INTRODUCTION

MicroRNAs (miRs) are a family of small, 20–25-nucleotide-long noncoding RNAs with the ability to post-transcriptionally downregulate the expression of specific target genes. At least a thousand miRs are predicted to operate in humans, regulating multiple physiological and pathological cellular processes. Bioinformatic predictions indicate that miRs are expected to regulate the expression of around 50% of all protein-coding genes, thus controlling the activity of a large fraction of the entire transcriptome (Inui *et al.*, 2010). miR biogenesis and action mechanisms have been extensively reviewed in the study by Bartel, (2009). miR-mediated gene expression control is achieved by specific base pair binding to the 3′UTRs of their target mRNAs, causing either mRNA degradation or translation inhibition (Filipowicz *et al.*, 2008; Bartel, 2009; Inui *et al.*, 2010). A single 3′UTR can contain numerous different miR binding sites, and therefore the crucial impact miRs have on biological processes is mainly because of the fact that each miR can simultaneously regulate several targets with disparate functions, making them fundamental regulators of tumor onset, growth, and progression (Inui *et al.*, 2010).

Cancer cells are characterized by peculiar hallmarks acquired during the multisteps of tumor progression. These include sustained proliferation, resistance to cell death, metabolism reprograming, host microenvironment interactions, angiogenesis, invasion, and seeding to distant organs (Hanahan and Weinberg, 2011). miR deregulation has a crucial role in cancer as it interferes with these specific hallmarks meaning miRs can either act as oncogenes or tumor suppressors in the early disease stages (Croce, 2009) or control the invasive and metastatic progression of cancer in the latter. Examples of miRs that control malignancy are miR-10b, miR-373/520c, miR-9 (pro-metastatic miRs), miR-335, the miR-200 family, and miR-31 (anti-metastatic miRs; Ma *et al.*, 2007; Huang *et al.*, 2008; Korpal *et al.*, 2008; Tavazoie *et al.*, 2008; Valastyan *et al.*, 2009; Ma *et al.*, 2010b). Relevantly, certain miRs are involved in specific tumor types, for instance the let-7 family, miR-34 family, miR-137, miR-182, miR-221/222, and miR-214 control melanoma progression (Mueller and Bosserhoff, 2009; Penna *et al.*, 2011).

This review focuses on the role of miR-214 in tumor progression. miR-214 is involved in numerous physiological and pathological processes including several cancers. The human miR-214 gene is located in the chromosomal region 1q24.3, in intron 14 of the Dynamin-3 gene (*DNM3*). As shown in Figure 1, an almost 8 kb-long noncoding RNA, named *DNM3os,* originates from intron 14 of *DNM3* in humans and mice. This transcript contains the sequences for miR-214 and miR-199a-2, two clustered miRs that are approximately 6 kb apart. Another small RNA sequence, that of miR-3120, is also present in this genomic region; however, it is on the reverse strand (Loebel *et al.*, 2005; Watanabe *et al.*, 2008; Lee *et al.*, 2009; Yin *et al.*, 2010; Scott *et al.*, 2012).

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Abbreviations: DNM3, Dynamin-3 gene; miR, microRNA

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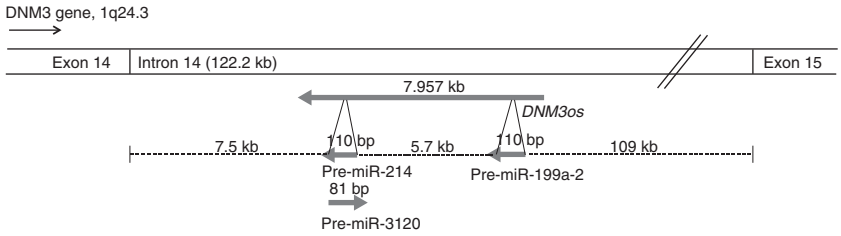

Figure 1. Genomic location of human miR-214. miR-214 and miR-199a-2 are 6 kb apart, located inside the 7.9 kb-long noncoding RNA precursor, *DNM3os*, in intron 14 of the Dynamin-3 (DNM3) gene (1q24.3). miR-3120 overlaps the miR-214 locus but in its complementary strand.

Twist-1, ZEB1, Ezh2, NFkB, and SMAD4 have been reported to control miR-214 and/or *DNM3os* transcription (Kong *et al.*, 2008; Juan *et al.*, 2009; Lee *et al.*, 2009; Yin *et al.*, 2010; Duan *et al.*, 2012; Williams *et al.*, 2012). miR-214 is highly conserved among species, which indicates that it is involved in broad physiological functions. In fact, it coordinates the cell fate, differentiation and morphogenesis of muscles, the skeleton, nervous system, retina, and pancreas (Flynt *et al.*, 2007; Joglekar *et al.*, 2007; Decembrini *et al.*, 2009; Chen *et al.*, 2010a; Shi *et al.*, 2013). High miR-214 expression is visible early in mouse embryogenesis in the heart, cerebellum, midbrain, nasal process, and limb buds and reaches a peak at E12.5. It is downregulated after E15.5 and further decreases in postnatal tissues, although its expression is detected in some adult organs, including the lungs, heart, and kidneys (Watanabe *et al.*, 2008; Aurora *et al.*, 2012). Despite the fact that miR-214 and miR-199a-2 belong to the same cluster and are mostly co-expressed during development (Watanabe *et al.*, 2008), the large distance between them in the locus and their different seed sequences suggest distinct regulatory mechanisms and biological functions. Disruption of the entire mouse *DNM3os* locus leads to skeletal abnormalities and death within 1 month after birth (Watanabe *et al.*, 2008). However, ubiquitous miR-214-specific knockout mice are viable and fertile, while displaying heart defects and cardiac contractility loss following ischemia-reperfusion injury (Aurora *et al.*, 2012), whereas bone-specific miR-214-overexpressing mice show defective osteogenic differentiation and bone formation (Wang *et al.*, 2013c). A specific miR-199a knockout mouse model is, however, necessary to clarify the contributions of miR-214 and miR-199a and to determine whether the differences between *DNM3os* and miR-214-null mice are due to the combination of these two miRs or due to the specific functions of the long noncoding RNA.

If we consider the fact that embryonic stemness and differentiation programs are often reactivated during tumor progression, the hypothesis that miR-214 has a role in tumorigenesis is well-grounded.

MIR-214 DOUBLE-FACED DEREGULATION IN CANCER

miR-214 is deregulated in several human cancers and displays contrasting behavior, thus suggesting that cell context is crucial for miR-214 function (Table 1).

Cutaneous cancers

There are three main types of skin cancers; basal cell carcinoma, Squamous Cell Carcinoma (SCC), and malignant melanoma. miR-214 is often altered in these, but particularly in melanoma, which is the least common but often most fatal of dermatologic tumors.

Although normal melanocytes display low miR-214 levels, the expression of this small RNA is high in nevi, which present abnormal, although benign, melanocyte proliferation and frequently contain genetic mutations (Grichnik, 2008; Glud *et al.*, 2009; Philippidou *et al.*, 2010; Chen *et al.*, 2010b; Penna *et al.*, 2011). Importantly, miR-214 is highly expressed in primary malignant cutaneous melanomas (Molnar *et al.*, 2008; Segura *et al.*, 2010; Penna *et al.*, 2011, 2013), although showing low expression levels in *in situ* noninvasive melanomas (Penna *et al.*, 2011). Moreover, miR-214 correlates significantly more with acral than with non-acral melanoma subtypes (Chan *et al.*, 2011) and is heavily expressed and associated with poor prognosis and elevated metastatic risk in ocular melanomas (Worley *et al.*, 2008). However, miR-214 levels may be slightly lower in metastases than in primary tumors, thus suggesting that this miR-214 increase has an important role at the time of radial to vertical/invasive melanoma growth (Penna *et al.*, 2011). Nevertheless, miR-214 upregulation in melanoma metastases is also associated with longer post-recurrence survival (Segura *et al.*, 2010). It is also important to note that miR-214 resides in a genomic region that is frequently amplified in melanoma, as demonstrated by Comparative Genomic Hybridization (CGH) in the study by Zhang *et al.*, (2006), whereas no reports indicate any correlation between miR-214 expression and typical melanoma genetic lesions, such as BRAF or KRAS mutations, to our knowledge. miR-199a-2, located in the same cluster as miR-214, is also amplified (Zhang *et al.*, 2006) and deregulated (Pencheva *et al.*, 2012) in melanoma. Patients with high miR-199a-3p and miR-199a-5p levels in primary melanomas show shorter metastasis-free survival compared with patients expressing low levels of these miRs (Pencheva *et al.*, 2012). Accordingly, high miR-199a levels in ocular melanomas correlate with metastatic risk (Worley *et al.*, 2008). As described in the following sections, miR-214 and miR-199a's involvement in driving melanoma metastasis has been clearly demonstrated (Penna *et al.*, 2011; Pencheva *et al.*, 2012; Penna *et al.*, 2013).

Although miR-214 is well expressed in epidermal keratinocytes and hair follicles and is relevant in skin morphogenesis (Ahmed *et al.*, 2012), contrasting observations have been reported regarding SCC and basal cell carcinoma. In fact, miR-214 is upregulated in oral and tongue SCC (Scapoli *et al.*, 2010; Yu *et al.*, 2010), whereas it was found to be significantly

Table 1. MicroRNA-214 alterations in cancer

Tumor	miR-214 expression in tumor vs. normal tissue	miR-214 correlation with metastasis or poor prognosis	Validated miR-214 targets	References
Melanoma	Up Down	Yes Yes	TFAP2, ITGA3	Penna *et al.*, 2011 Worley *et al.*, 2008 Segura *et al.*, 2010
Pancreas	Up	—	ING4	Zhang *et al.*, 2010 Volinia *et al.*, 2006
Stomach	Up	Yes	PTEN	Volinia *et al.*, 2006 Ueda *et al.*, 2010 Yang *et al.*, 2013
Prostate	Up	—	—	Volinia *et al.*, 2006
Nasopharygeal	Up	—	LTF, BIM	Deng *et al.*, 2013 Zhang *et al.*, 2014
Oral/tongue squamous	Up	—	—	Yu *et al.*, 2010 Scapoli *et al.*, 2010
Osteosarcoma	Up	Yes	LZTS1	Wang *et al.*, 2014c Xu and Wang, 2014
T-cell lymphoma	Up	Yes	—	Narducci *et al.*, 2011
Lung	Up	—	—	Yanaihara *et al.*, 2006
Hepatocellular	Down	Yes	HDGF, XBP1, EZH2, FGFR1, β-catenin	Shih *et al.*, 2012 Xia *et al.*, 2012 Wang *et al.*, 2013b
Uterus cervix	Down	Yes	PLXNB1, GALNT7, MEK3, JNK1	Yang *et al.*, 2009 Qiang *et al.*, 2011
Adrenocortical	Down	—	—	Tombol *et al.*, 2009
Esophageal	Down	—	—	Huang *et al.*, 2012
Cutaneous squamous	Down	—	ERK1	Yamane *et al.*, 2013
Myeloma	Down	—	ASF1B, GANKYRIN	Gutierrez *et al.*, 2010
Bladder	Down	Yes	—	Ratert *et al.*, 2013
Glioma	Down	Yes	UBC9	Wang *et al.*, 2014b
Colorectal	Down	Yes	FGFR1	Chen *et al.*, 2014
Rhabdomyosarcoma	Down	—	NRAS	Huang *et al.*, 2014
Ovary	Up Down	Yes Yes	PTEN, p53	Yang *et al.*, 2008 Marchini *et al.*, 2011 Iorio *et al.*, 2007
Breast	Up Down	Yes Yes	EZH2	Derfoul *et al.*, 2011 Schwarzenbach *et al.*, 2012 Blenkiron *et al.*, 2007

MicroRNA-214 (miR-214) altered expression (up- or downregulation) in different types of human cancers. Where possible, the correlation with metastasis/poor prognosis and malignancy-involved validated targets is indicated.

downregulated in esophageal and cutaneous SCC, leading to abnormal keratinocyte proliferation (Huang *et al.*, 2012; Yamane *et al.*, 2013). miR-214 expression seems not to be involved in basal cell carcinoma, however (Sand *et al.*, 2012).

Various non-cutaneous tumors

miR-214 is upregulated in pancreatic (Volinia *et al.*, 2006; Zhang *et al.*, 2010), prostate (Volinia *et al.*, 2006), stomach (Volinia *et al.*, 2006; Ueda *et al.*, 2010; Li *et al.*, 2011; Yang *et al.*, 2013; Wang *et al.*, 2014a), nasopharyngeal (Deng *et al.*, 2013; Zhang *et al.*, 2014), and lung (Yanaihara *et al.*, 2006) tumors, as well as in osteosarcomas (Xu and Wang, 2014; Wang *et al.*, 2014c) and in a leukemic variant of cutaneous T-cell lymphomas, the *Sezary syndrome* (Narducci *et al.*, 2011), as compared with healthy tissues. High miR-214 expression has been associated with unfavorable prognosis in overall survival and clinical staging, invasiveness, metastasis, and poor response to therapy (Ueda *et al.*, 2010; Narducci *et al.*, 2011; Yang *et al.*, 2013; Wang *et al.*, 2014c). Conversely, noteworthy miR-214 downregulation occurs in hepatocellular (Duan *et al.*, 2012; Shih *et al.*, 2012; Wang *et al.*, 2012a, 2013b), uterus cervix (Yang *et al.*, 2009), adrenocortical (Tombol *et al.*, 2009), bladder (Ratert *et al.*, 2013), colorectal (Chen *et al.*, 2014), and cholangio (Li *et al.*,

2012) carcinomas, as compared with healthy tissues, as well as in multiple myelomas (Gutierrez *et al.*, 2010), gliomas (Wang *et al.*, 2014b), and in lung adenocarcinoma-derived brain metastases (Zhao *et al.*, 2013). In these neoplasias, miR-214 reduction is often associated with malignancy, metastasis, or poor survival/recurrence (Qiang *et al.*, 2011; Li *et al.*, 2012; Xia *et al.*, 2012; Ratert *et al.*, 2013; Wang *et al.*, 2013b; Chen *et al.*, 2014).

miR-214 displays more complex behavior in breast and ovary cancers, possibly due to high tumor heterogeneity and hormone responsiveness. The miR-214 locus is frequently amplified in these tumors, as shown by CGH (Zhang *et al.*, 2006); however, it has been found to have been deleted in 24% of a small group of breast cancer cases (Derfoul *et al.*, 2011). miR-214 is less expressed in large cohorts (Dvinge *et al.*, 2013) of breast tumor samples than in normal tissues, whereas its upregulation has been found in Luminal A, Normal-like and ER-,PR-,HER2 +/− breast cancer subtypes (Blenkiron *et al.*, 2007; Sempere *et al.*, 2007). Similarly, miR-214 is overexpressed in ovary cancers and significantly associated with high grade and late/metastatic tumor stages, as well as with overall and progression-free survival and post-surgical/chemotherapy recurrence (Yang *et al.*, 2008; Marchini *et al.*, 2011). However, miR-214 downregulation has also been described in neoplastic, as compared with normal, ovaries, as well as in ovary cancer–derived effusions as compared with primary tumors (Iorio *et al.*, 2007; Vaksman *et al.*, 2011).

These data, taken as a whole, suggest that miR-214 deregulation could have a relevant impact on malignancy, even if it can operate in opposite manners in different tumors. Therefore, it should be evaluated for diagnostic/therapeutic purposes.

CIRCULATING MIR-214 AS A DIAGNOSTIC MARKER

Interestingly, miR-214 has been observed to be a circulating miR, and its serum levels have shown diagnostic potential. In fact, analyses of miR-214 levels in the serum of over 100 breast cancer patients were able to discriminate between malignant and benign tumors, as well as healthy controls, whereas levels were significantly decreased in post-operative sera but increased in association with metastatic spread to regional lymph nodes (Schwarzenbach *et al.*, 2012). miR-214 blood serum expression was also able to identify patients carrying malignant peripheral nerve sheath tumors (Weng *et al.*, 2013). Similarly, the analysis of circulating exosomes isolated from ovarian carcinoma patients revealed the presence of miR-214 (overexpressed in the primary tumor mass), together with 7 other miRs, which strongly supports the case for miR-214's diagnostic power (Taylor and Gercel-Taylor, 2008). The same situation was observed when exosomes from lung cancer patients were analyzed (Rabinowits *et al.*, 2009). Furthermore, urinary miR-214 has been proven to be a diagnostic biomarker for bladder and prostate cancers (Kim *et al.*, 2013; Srivastava *et al.*, 2013). These observations reinforce the potential of extracellular miR-214 use as a diagnostic or prognostic biomarker.

MIR-214 FUNCTIONS AND TARGETS IN TUMOR PROGRESSION

Altered miR-214 expression in cancer has led numerous researchers to investigate its tumor-specific functions and to identify its targets in the various tumor formation and progression steps. miR-214's pleiotropic roles and direct targets are summarized in Figure 2 and discussed below.

Proliferation, cell cycle, and survival

Altered levels of miR-214 in tumors seem to hint at a possible influence on proliferation and cell cycle control; however, existing data are controversial. No positive miR-214 cell viability or growth regulation was observed in melanomas (Penna *et al.*, 2011), lung (Fei *et al.*, 2008), or pancreatic (Zhang *et al.*, 2010) carcinomas. By contrast, miR-214 was found to promote AKT signaling, proliferation, and survival in gastric tumors via PTEN and in nasopharyngeal carcinomas via lactoferrin and Bim targeting (Deng *et al.*, 2013; Yang *et al.*, 2013; Zhang *et al.*, 2014), and to favor osteosarcoma growth via LZTS1 repression (Xu and Wang, 2014). On the other hand, miR-214 inhibits proliferation in breast or hepatic carcinomas via Ezh2, β-catenin, and XBP1 (an ER-stress-induced pro-survival factor) targeting; miR-214 expression also reduces hepatoma xenograft growth (Derfoul *et al.*, 2011; Duan *et al.*, 2012; Shih *et al.*, 2012; Xia *et al.*, 2012; Wang *et al.*, 2012a). Furthermore, proliferation is decreased by miR-214 in rhabdomyosarcomas via N-Ras oncogene suppression (Huang *et al.*, 2014), in cervical cancers via MEK3 and JNK1, GALNT7 and Plexin-B1 downregulation (Cheng *et al.*, 2005; Yang *et al.*, 2009; Qiang *et al.*, 2011; Peng *et al.*, 2012), and in squamous cell carcinoma cells via ERK1 inhibition (Yamane *et al.*, 2013). In myelomas, miR-214 targets ASF1B and gankirin, thus inducing p53, p21, and Bax upregulation and resulting in the inhibition of DNA replication and apoptosis (Misiewicz-Krzeminska *et al.*, 2013). A link between miR-214 and Receptor Tyrosine Kinases (RTKs) was also observed. In fact, miR-214 targets FGFR1 in hepatocellular and colorectal carcinomas, thus influencing cell growth (Wang *et al.*, 2013b; Chen *et al.*, 2014), and the ErbB3 cis-interactor Necl-2, leading to enhanced ErbB2/3 signaling in colon carcinomas (Momose *et al.*, 2013).

Angiogenesis

Tumor vascularisation has a central role to play in cancer progression. Indeed, neo-angiogenesis within the tumor mass provides cancer cells with the blood vessels necessary for growth and, in the meanwhile, facilitates the intravasation and dissemination of metastatic cells. On the other hand, hypoxic conditions, caused by insufficient or aberrant vascularization, often contribute to the selection of more malignant tumor cell subpopulations (Carmeliet and Jain, 2011). Interestingly, miR-214 has a key role in angiogenesis. It is expressed in endothelial cells (Wurdinger *et al.*, 2008; Rippe *et al.*, 2012) and inhibits HUVEC angiogenesis and vascular network formation by targeting Quaking and, consequently, reduces the secretion of pro-angiogenic growth factors and cytokines, including VEGF, CCL5, and CXCL5 (van Mil *et al.*, 2012). In addition, miR-214 downregulates the eNOS (nitric oxide

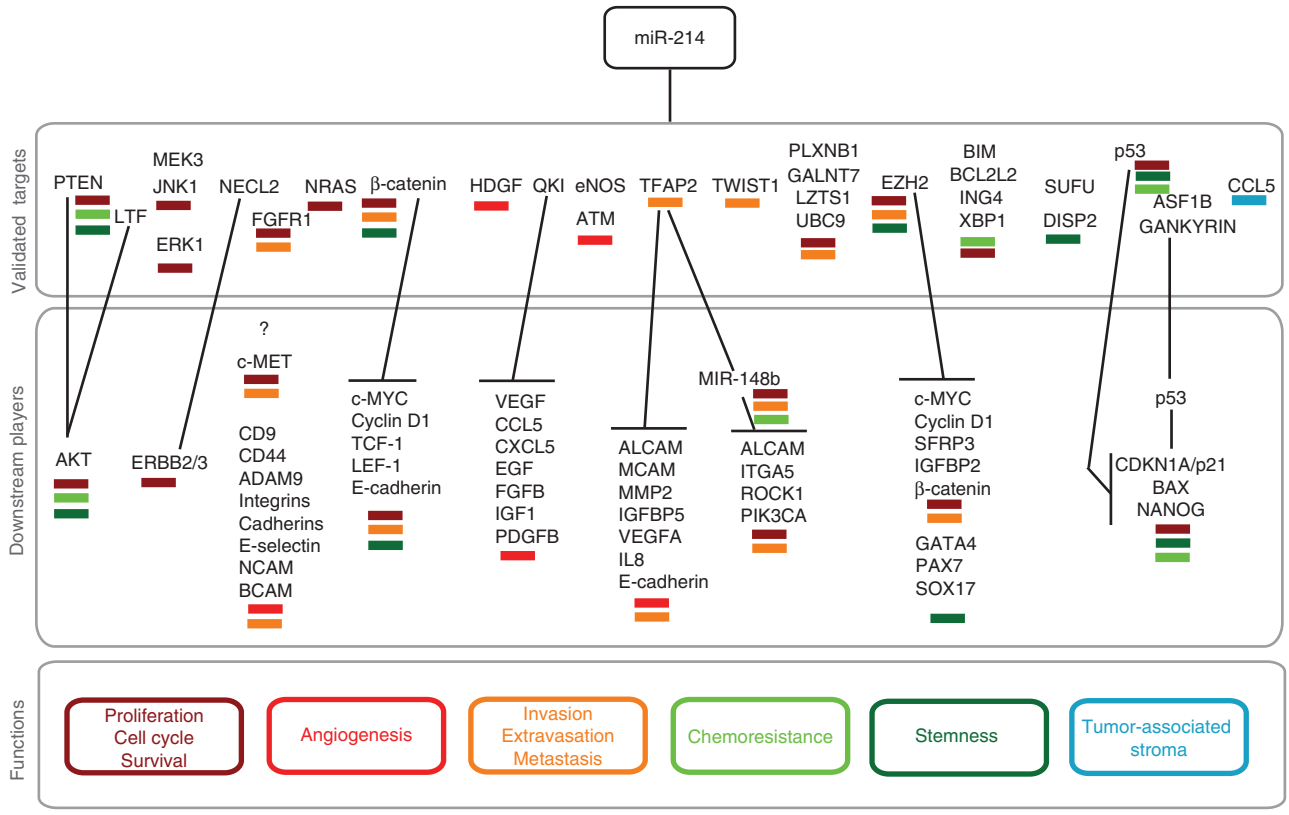

Figure 2. miR-214 controls cancer networks. miR-214 direct target genes and indirect downstream players are shown, together with their multiple functions in tumor cells (different colors).

synthase) enzyme, which is essential for endothelial cell migration (Chan *et al.*, 2009). By contrast, adult endothelial cells (HMEC-1) secrete miR-214-containing exosomes which, in turn, stimulate a pro-angiogenic program in neighboring cells via ATM downregulation (van Balkom *et al.*, 2013). Considering the cross talk between tumor and endothelial cells within a tumor mass, the existence of a pivotal role for miR-214 can be envisaged in tumor vascularization. Indeed, miR-214 downregulation in hepatocellular carcinoma contributes to tumor angiogenesis, whereas its ectopic overexpression reduces tumor growth and vascularity. This occurs via its direct target, hepatoma-derived growth factor (HDGF; Shih *et al.*, 2012), thus demonstrating that miR-214 has an anti-angiogenic role in hepatocarcinomas, in which miR-214 downregulation is associated with worse clinical outcome.

Migration, invasion, extravasation, and metastasis

miR-214 upregulation in melanoma cell lines significantly promotes tumor cell migration, invasion, adhesion to extracellular matrices, transendothelial migration, and survival to anoikis *in vitro*, as well as extravasation from blood vessels and metastasis formation in mice. This occurs without affecting cell proliferation and tumor growth via the simultaneous coordination of a network including over 70 genes (Penna *et al.*, 2011). In particular, miR-214 acts by directly targeting transcription factors TFAP2, well-established melanoma tumor suppressors, and upregulating ALCAM, an adhesion receptor known as a melanoma progression marker. ALCAM upregulation occurs via TFAP2 and miR-148b, a small RNA that is downregulated by miR-214. Rescuing experiments have demonstrated that TFAP2 or miR-148b downmodulation and ALCAM upregulation control miR-214-dependent melanoma metastatic traits and metastasis formation (Penna *et al.*, 2011, 2013), thus highlighting the importance of a critical pathway that involves these players. Importantly, several miR-148b target genes, including ALCAM, ROCK1, PIK3CA, and ITGA5, are upregulated by miR-214, because of miR-148b downmodulation, which could account for additional miR-214 pro-malignant functions (Penna *et al.*, 2013; Orso *et al.*, unpublished data). Moreover, the miR-214-dependent modulation of other genes in this network may influence melanoma progression, as well. These include genes that regulate adhesion, movement, invasion, and matrix degradation (integrins, cadherins, MCAM, NCAM, BCAM, PAK2, MMP2, ADAMs, TIMPs and the MET oncogene), others that are implicated in interactions with endothelial cells and extravasation (E-Selectin, CD9, IL-8, SEMA3A, VEGFR2, VEGFA and ALCAM itself), and still others that are members of the TGFβ, Wnt, and Notch pathways and that are potentially involved in the epithelial-to-mesenchymal transition. These findings appear to indicate a clear pro-invasive and pro-metastatic role played by miR-214 in melanoma. Nevertheless, other authors have observed, in a similar context, the existence of a significant role for the small RNA that is clustered

with miR-214, miR-199a-2. Indeed, both miR-199a-5p and miR-199a-3p have been demonstrated to strongly drive melanoma invasion and metastatic colonization and to enhance angiogenesis by synergistically targeting ApoE and DNAJA4 and thus controlling the LPR1 and LPR8 pathways (Pencheva *et al.*, 2012). It is possible that miR-214 and miR-199a operate in an alternative or synergistic manner in melanoma progression, and detailed experiments need to be performed to unravel the precise functions of these two clustered miRs.

miR-214 significantly encourages migration and invasion in gastric cancer by targeting PTEN (Yang *et al.*, 2013), whereas it favors osteosarcoma invasion by silencing LZTS1 (Xu and Wang, 2014), and, furthermore, nasopharyngeal carcinoma metastasis formation has been found to be promoted via lactoferrin repression in a mouse xenograft model (Deng *et al.*, 2013). miR-214 has been shown to display pro-invasive or pro-metastatic properties in mammary tumor cells (Penna *et al.*, 2011), although a deeper analysis of miR-214 functions in breast cancer is still pending. Indeed, Derfoul and colleagues have reported opposite data: decreased miR-214 levels in mammary carcinoma cells correlate with increased cell invasion via the accumulation of miR-214 target, Ezh2, a known marker of malignancy that coincides with the histone H3K27 trimethylation catalytic subunit of the Polycomb repressive complex 2, which epigenetically regulates chromatin and thereby influences gene expression (Derfoul *et al.*, 2011; Crea *et al.*, 2012). Similarly, miR-214 suppresses cell invasion by downregulating Ezh2, β-catenin, and FGFR1 in hepatocellular carcinoma cells; the high expression of these genes correlates with low miR-214 levels and increased malignancy (Xia *et al.*, 2012; Wang *et al.*, 2013b). Importantly, sustained β-catenin and/or Ezh2 expression in breast or hepatic tumors leads to malignancy as the levels of their downstream genes, such as c-Myc, cyclin D1, TCF-1, LEF-1, E-cadherin, SFRP3, IGFBP2, and even β-catenin itself, are affected (Derfoul *et al.*, 2011; Xia *et al.*, 2012; Wang *et al.*, 2012a). Interestingly, Ezh2 repression by miR-214 in esophageal squamous carcinoma cells also results in migration and invasion inhibition (Huang *et al.*, 2012). Furthermore, miR-214 has been shown to inhibit invasion via Plexin-B1 and GALNT7 targeting in cervical cancers (Qiang *et al.*, 2011; Peng *et al.*, 2012), via FGFR1 repression in colorectal tumors (Chen *et al.*, 2014) and via Twist-1 (a master regulator of metastasis) downregulation in intrahepatic cholangiocarcinomas (Li *et al.*, 2012).

Chemoresistance

Despite the controversial findings concerning the role of miR-214 in proliferation, one of the first miR-214 functions described was its ability to reduce apoptotic cell death, contributing to decreased chemotherapy sensitivity, the main cause of treatment failure and disease progression. In particular, miR-214 has been shown to inhibit cisplatin-induced apoptosis in human ovarian cancers primarily by targeting PTEN and enhancing the AKT pathway and survival (Yang *et al.*, 2008). Moreover, miR-214 has been proven to contribute to cisplatin chemoresistance in tongue squamous cell carcinomas (Yu *et al.*, 2010). Accordingly, miR-214 is significantly upregulated in gefitinib-resistant EGFR-mutant non-small cell-lung cancer cells. PTEN targeting also has a major role in this case. In fact, miR-214 knockdown is sufficient to normalize AKT pathway activation and to resensitize cells to the therapy (Wang *et al.*, 2012b). Furthermore, miR-214 overexpression in pancreatic cancer cells decreases sensitivity to gemcitabine, the first line treatment for advanced pancreatic cancers, probably via its direct target ING4, a gene involved in cell cycle arrest, DNA repair, and apoptosis (Zhang *et al.*, 2010). Moreover, high miR-214 levels have been associated with poor chemotherapy response in pediatric osteosarcomas (Wang *et al.*, 2014c) and with increased T-cell lymphoma cell survival and apoptotic resistance (Narducci *et al.*, 2011). Finally, miR-214 has been predicted to control paclitaxel sensitivity in laryngeal cancer cells (Xu *et al.*, 2013). The only study that has published contrary results was performed in cervical cancer cells where miR-214 enhances cisplatin-induced cytotoxicity, via the downregulation of Bcl2l2 (Wang *et al.*, 2013a). In melanomas, miR-214 reduces apoptosis and facilitates survival in adhesion-lacking (anoikis) conditions (Penna *et al.*, 2011), which would seem to suggest a potential role in chemoresistance; however, the details of its actual involvement are still to be explored.

Cancer stemness

miR-214 has a key role in normal cell differentiation by targeting Ezh2 (see above), which leads to the consequent derepression of the master developmental regulators Gata-4, Pax7, and Sox17 (Juan *et al.*, 2009). As developmental pathways are often reactivated during tumorigenesis and stemness markers are observed in tumor stem cells (Nguyen *et al.*, 2012), the involvement of miR-214 in cancer stem cells has been investigated and confirmed. In fact, miR-214 has been shown to regulate ovarian cancer stem cell properties by directly repressing p53 and indirectly upregulating Nanog, leading to increased stem cell population and self-renewal (Xu *et al.*, 2012). However, lower miR-214 expression has been reported in ovarian cancer stem cells than in mature cells, and here the miR-214-controlled PTEN-AKT pathway is seen to be a major regulator of stemness (Yin *et al.*, 2010). Interestingly, miR-214 was found to be the most highly expressed of a list of 37 differentially expressed small noncoding RNAs in an analysis of miR expression in purified $CD44^+CD24^-lin^-$ breast cancer stem cells (Shimono *et al.*, 2009). miR-214 expression has also been found to be significantly increased in pancreatic cancer cell–derived holoclones, which are capable of long-term survival, tumor initiation, and chemoresistance (Tan *et al.*, 2011). In contrast, miR-214 has been demonstrated to suppress stem-like traits in human hepatocellular carcinoma cells by directly targeting Ezh2 and β-catenin signaling pathways (Xia *et al.*, 2012). These findings indicate that miR-214 has contrasting functions in controlling cancer stemness, depending on its role in specific malignancies.

Tumor-associated stroma

Cancer-associated fibroblasts (CAFs) have a major role in the tumor microenvironment; however, the conversion of quiescent fibroblasts into CAFs is still quite a mystery. miR-214 is

relevant in this process as ovarian cancer cells, in fact, induce miR-214 downregulation in normal tumor-adjacent fibroblasts, thus reprograming them into CAFs with increased chemokine secretion. A particular example is CCL5, which is a direct miR-214 target (Mitra *et al.*, 2012). On the other hand, miR-214 is upregulated in gastric cancer–derived mesenchymal stem cells, which are involved in invasion (Wang *et al.*, 2014a).

CONCLUDING REMARKS

In conclusion, miR-214 appears to be a pleiotropic hub that participates in the control of cancer cell signaling networks. In fact, it contributes to the coordination of essential signaling pathways, such as the PTEN/AKT, β-catenin, or receptor tyrosine kinase pathways, as well as crucial gene expression modulators, including Ezh2, p53, TFAP2, and miR-148b. The fact that miR-214 displays specific and even contrasting functions in different tumor types is most likely due to differential mRNA targeting and/or target gene expression in different cancer cell contexts. In particular, as miR-214 broadly controls gene expression by regulating transcription factors and other miRs, disparities in the expression of subsets of these downstream players in different tumors, or tumor cell populations, may account for the versatility of miR-214 actions. Thus, miR-214 may have contrasting roles according to co-expression with its own direct or indirect target genes or with other miRs (for instance miR-148b or the clustered miR-199a-2) and their targets. This may also explain the switch between proliferation/survival phenotypes in certain cancers to movement/invasion traits in others. Importantly, some known miR-214-regulated genes (e.g., TFAP2 and ALCAM) display contrasting behavior in different tumor types in which miR-214 also has contrasting functions. For instance, although TFAP2 factors are well-defined tumor suppressors in melanoma and are often lost during disease progression (Bar-Eli, 2001), their role in breast tumors is more complex. In fact, TFAP2A is frequently upregulated in ErbB2/HER2-positive breast carcinomas and induces ErbB2/HER2 transcription (Bosher *et al.*, 1996; Turner *et al.*, 1998; Shiu *et al.*, 2014). However, other studies have observed reduced TFAP2 levels in high grade and metastatic breast cancers (Pellikainen *et al.*, 2002; Sotiriou *et al.*, 2006). Moreover TFAP2C overexpression in a MMTV/Neu transgenic mouse model resulted in reduced tumorigenesis (Jager *et al.*, 2005). Similarly, high ALCAM positivity is a clinical marker for melanoma thickness and invasiveness (van Kempen *et al.*, 2000). ALCAM upregulation, as opposed to normal mammary tissue, has furthermore been observed in intraductal/invasive breast carcinomas (Burkhardt *et al.*, 2006) and correlates with metastasis, as well (Piao *et al.*, 2012). However, low ALCAM levels have also been associated with mammary nodal infiltration, metastatic dissemination, and worse clinical outcome (King *et al.*, 2004; Jezierska *et al.*, 2006).

Tumor mouse models are essential to an improved definition of miR-214 involvement in cancer, and as such other tissue-specific miR-214-deleted or miR-214-overexpressing mice need to be established and analyzed for this purpose, in addition to existing animal models (Aurora *et al.*, 2012; Wang *et al.*, 2013c). Additional investigations must carefully evaluate miR-214 genomic locus and transcriptional/post-transcriptional events if we want to understand how miR-214 deregulation occurs in cancer. Furthermore, circulating miR-214 has been proven to be a potential biomarker (Schwarzenbach *et al.*, 2012), and therefore its diagnostic/prognostic value should be explored in depth and in various tumors. Finally and more importantly, as miR-based *in vivo* therapy attempts have already been successful (Care *et al.*, 2007; Elyakim *et al.*, 2010; Wiggins *et al.*, 2010; Ma *et al.*, 2010a), the potential use of miR-214 as a therapy target merits careful investigation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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