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Comprehensive Patterns in MicroRNA Regulation of Transcription Factors During Tumor Metastasis

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ABSTRACT

In spite of a large body of information about the upstream regulators of metastasis, a process that often plays a limiting factor in therapeutic outcome of cancer patients, the impact of regulatory microRNA patterns remains obscure. This review describes computational analysis of coordinated regulation of genes by di-directional regulation of microRNA and transcription factors that specifically regulate the process of metastasis. We discovered several unexpected modes of regulatory patterns between microRNAs and transcription factors. For example, we found a double positive feedback loop regulated by the hub transcription factor ZEB1 and miR-200 during epithelial–mesenchymal transition. This review further explains flow of information and how such components coordinate various adaptable controls of microRNAs and thus, contribute to regulation of transcription factors in context of cancer metastasis. Information described here provides a regulatory framework for future experimental analyses and discoveries of new insights into post-transcriptional gene regulation at the microRNA level in cancer metastasis. J. Cell. Biochem. 112: 2210–2217, 2011.

KEY WORDS: MICRORNAS; TRANSCRIPTION FACTOR; METASTASIS; FEED FORWARD LOOP; FEED BACK LOOP

The major reasons for over 90% cancer deaths are complications arising from metastasis and hence improved treatment will require targeting metastatic disease in addition to the primary tumor [Hunter et al., 2008]. Studies on the molecular mechanisms of cancer, have demonstrated existence of at least a subset of tumors characterized by over-dependence on certain key regulatory networks. Examination of mRNA expression patterns have often yielded conflicting results related to specific roles in metastasis, prompting questions on the actual existence of a specific metastasis transcriptome. Evidence shows that microRNAs and Transcription factors (TFs) are complex trans-acting molecules that activate or repress genes through *cis*-regulatory binding sites [Bommer et al., 2007; Woods et al., 2007; Schulte et al., 2008] and recent studies experimentally validate the role of numerous transcriptomal loops involving microRNAs and TFs that have been found in a variety of organisms [Johnston et al., 2005; Fazi et al., 2007; Kim et al., 2007; Varghese and Cohen, 2007].

Transcription factors (TFs) and microRNAs are prime members of trans-acting gene regulatory molecules in organism development, function, and disease. Both TFs and microRNAs are trans-acting factors that exert their activity through composite *cis*-regulatory elements. By binding to distinct *cis*-regulatory elements, individual TFs and microRNAs can control large number of target genes. While TFs physically interact with *cis*-regulatory DNA elements to activate or repress transcription of their target genes, microRNAs repress gene expression post-transcriptionally by interacting with complementary sequences located in the 3'-untranslated region (UTR) of their target mRNAs [Bartel, 2004; Hobert, 2008]. This implies that microRNAs and TFs could be intricately connected in the networks that control differential gene expression of an organism. The collaborative regulation of microRNAs and TFs is reflected at the transcriptional and post-transcriptional levels of cellular complexity [Wang and Purisima, 2005; Batada et al., 2006]. Recently, mapped transcriptional regulatory networks of protein-coding genes have been described in yeast [Harbison et al., 2004], *C. elegans* [Deplancke et al., 2006; Vermeirssen et al., 2007], *Drosophila melanogaster* [Sandmann et al., 2006], and mammals [Boyer et al., 2005]. Although such data are incomplete, these networks have provided insights into overall network architecture and also revealed particular network subgraphs that were over-represented in real networks compared with randomized networks [Milo et al., 2002; Shen-Orr et al., 2002].

In gene regulatory circuits, TFs and microRNA may reciprocally regulate one another to form feedback loops, or alternatively, both TFs and microRNA may regulate their target genes and form feed-forward loops (FFLs). FFLs in regulatory circuits offer a mechanism

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to provide a robust transcriptional response to signals and provide protection against transcriptional noise [Milo et al., 2002; Shen-Orr et al., 2002]. FFLs seem to be over-represented in pure transcription regulatory networks whereas feedback loops were found to be less abundant. Yeger-Lotem et al. give an explanation for the scarcity of feedback loops in that they may be generated by a combination of transcriptional and post-transcriptional mechanisms, as opposed to being purely transcriptional [Shen-Orr et al., 2002; Yeger-Lotem et al., 2004]. Two recent studies explored hundreds of potential microRNA-mediated feedback and FFLs at the genome level in mammals and found interesting regulatory motifs [Shalgi et al., 2007; Tsang et al., 2007]. Feedback loops are important in homeostasis and cellular differentiation programs [Alon, 2007]. FFL is an important regulatory motif and has been found in organisms such as *Escherichia coli*, yeast, and humans [Mangan et al., 2003]. Since microRNAs play key regulatory functions in gene expression, a FFL consisting of a transcription factor, microRNA, and a target gene will be a powerful tool to investigate regulatory mechanisms in disease at both transcriptional and translational levels. Presently, only a few FFL have been experimentally verified and include E2Fs-Myc-miR-17/20 [Sylvestre et al., 2007], E2F-miR-106b/93/25-CDK inhibitors [Brosh et al., 2008], and PKC-MAPK-miR-15a [Cohen et al., 2009]. Composite feedback loop of a microRNA-transcription factor has served as a direct regulatory motif and a few experimental examples are ZEB1/SIP1 and miR-200 family in embryologic development and Pitx3-miR-133b in neuron development [Kim et al., 2007; Bracken et al., 2008]. Recent bioinformatics studies propose that correlations and anti-correlation of microRNAs and their targets occurs due to various types of feed-forward and feedback loops involving microRNAs, their predicted target genes, and upstream regulators like TFs and kinases [Farh et al., 2005; Stark et al., 2005; Sood et al., 2006; Tsang et al., 2007].

Our goal therefore is to focus on detailed mechanistic insights on key regulators of tumour metastasis that may eventually prove to be interesting diagnostic and/or therapeutic targets in cancer. We have compiled inclusive patterns of miRNA-transcription factor regulatory network of tumor metastasis in different cancer types. Furthermore, we computationally explored potential miR-TF patterns in major effector mechanisms of cervical cancer progression (namely invasion, extra cellular matrix (ECM) modification, epithelial–mesenchymal transition (EMT), and angiogenesis). This then provides a comprehensive catalog of miR-TF regulatory patterns that regulate tumor progression and metastatic processes.

MICRORNA/TRANSCRIPTION FACTOR-MEDIATED REGULATORY PATTERNS IN TUMOR METASTASIS

Metastasis involves multiple steps which are often selective and rate limiting. These include invasion, intravasation, circulation, extravasation, and metastatic colonization [Chambers et al., 2002; Leber and Efferth, 2009]. Despite significant advances in metastasis research, the molecular patterns involved in these mechanisms are incompletely understood. In recent years, investigations allowed identification of microRNA abnormalities in human cancer by analyzing their transcriptional regulators. We therefore systematically investigated connections between transcriptional and post-transcriptional network interactions in tumor metastasis. We first searched the Tumor Suppressor and Oncogene Directory (http://embryology med.unsw.edu.au/DNA/DNA10.htm) and also did a manual curation of literature to develop a cataloge of mixed FFLs and feedback loops in which a master transcription factor regulates a microRNA and, together with it, a set of joint target protein-coding genes. The lists of FFLs and Feedback loops in metastasis process demonstrate a connection between such loops and various molecular regulons.

COHERENT TYPE II FFLs ARE THE FIRST LINE OF DEFENSE

In the coherent Type II FFL, a transcription factor or gene X regulates transcription factor Y, and both then jointly regulate gene Z. In the context of a metastasis associated event, 11 microRNAs were found to be involved in extracellular matrix remodeling and constituting 15 coherent type II FFLs. The process of invasion is regulated by 19 type II coherent FFLs and angiogenesis by 3 FFLs.

In studies on breast cancer metastasis, Huang et al. showed that miR-373 and miR-520c mediate their invasive and metastatic effects partly through direct suppression of CD44 through a coherent Type II FFL [Qihong et al., 2008] (Fig. 1). miR-21 has emerged as a major microRNA that is overexpressed during invasion. It also down-regulates tropomyosin 1 (TPM1), that bind to the sides of actin filaments post-transcriptionally through a coherent Type II FFL thereby stimulating invasion and metastasis [Zhu et al., 2007].

The expression of microRNAs such as miR-335, miR-206, and miR-126 was inversely associated with metastatic relapse of tumors to distant organs through SOX4 and TNC organizing multiple coherent Type II FFLs that are specifically required for extracellular matrix modification as well as invasion [Tavazoie et al., 2008]. Another set of coherent Type II FFLs was constructed by transcription factor encoding leucine zipper proteins such as FOSL2 with their target microRNAs like miR-27, miR-30, miR-106, miR-17, and miR-20a. The transcription factor STAT3 (Signal Transducer and Activator of Transcription) is targeted by multiple microRNAs, miR-106, miR-20a, miR-182, miR-124, miR-20b, miR-17, and miR-124 forming a network of microRNA-TF coherent Type II FFLs (Fig. 1). CCAAT/enhancer-binding protein (CEBP) is a potential early biomarker in pancreatic neoplasia that is targeted by miR-155 constituting another efficient system of coherent Type II FFL [Koshiol, 2010]. Another coherent type II FFL is formed by MiR-10b which is upregulated in breast cancer metastasis exerting its effects through the transcription factor HOXD10 [Ma et al., 2007]. MiR-26, miR-186, and p53 microRNAs such as miR-126 and miR-181 express their metastasis associated activity through hHLHB2 a major transcription factor constitutes a coherent type II FFL [Boominathan, 2009]. Studies by Feng et al. [2008] also showed the association of miR-126 expression level with tumour size, lymph node metastasis, local invasion, epithelial mesenchymal transition, and node metastasis.

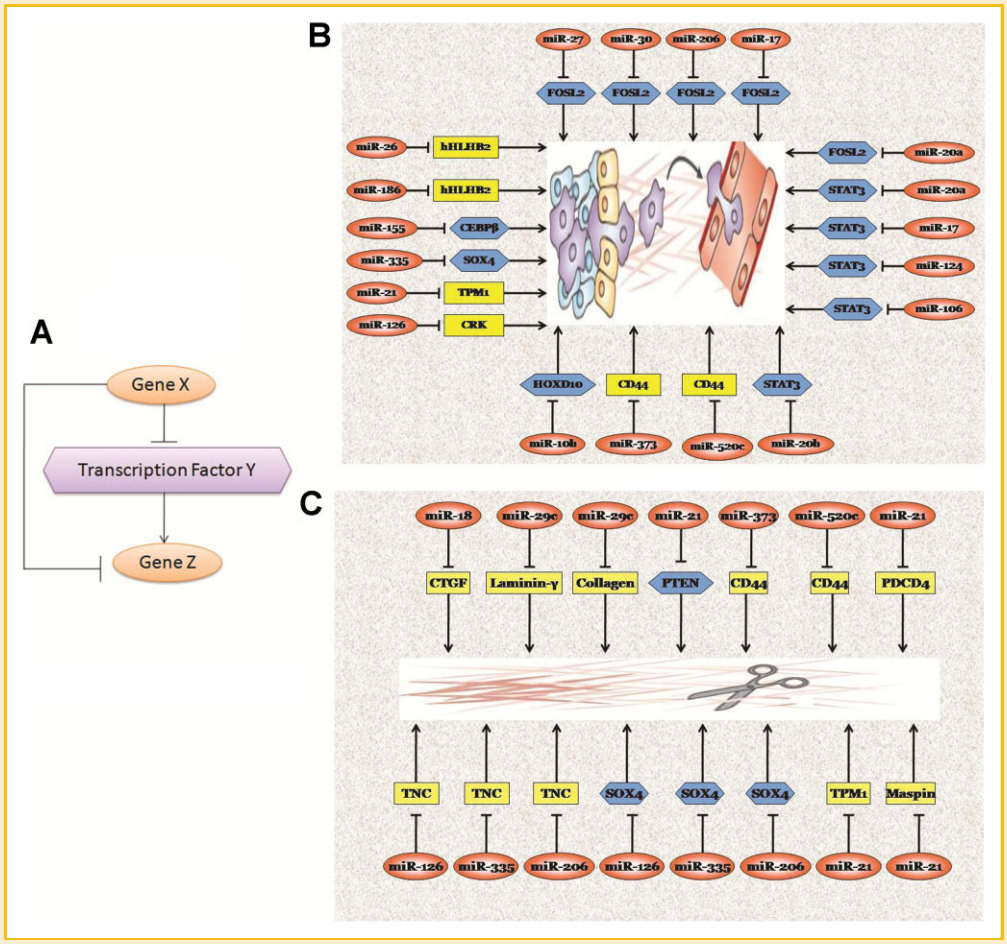

Fig. 1. A: Coherent type II FFL, (B) coherent type II FFLs in invasion, and (C) coherent type II FFLs in extracellular matrix modification.

MiR-29c suppresses extracellular matrix modification through collagens and laminins and repressed transcriptionally through MYC [Feng et al., 2008]. The same coherent type II FFL which operates in invasion between miR-373 and miR-520c on CD44 [Qihong et al., 2008], miR-126 and miR-335 and miR-206 on SOX4 and TNC [Tavazoie et al., 2008] as well as miR 21 on TPM1 [Zhu et al., 2007] are also found to regulate extracellular matrix modification (Fig. 1). The miR-21 targets include the tumour suppressor phosphatase and tensin homolog (PTEN), a transcription factor that functions as a dual specificity phosphatase and also serves as an actin-binding protein and stabilizes microfilaments. It is often mutated in a variety of tumours and is involved in the AKT survival pathway central to tumor development [Zhu et al., 2007]. The downregulation of tropomyosin1 promotes epithelial cell transformation into a mesenchymal phenotype and promotes invasion [Boyd et al., 1998]. The many targets of miR-21 constitute a delicate coherent Type II network that regulates tumor metastasis and include maspin and PDCD4, an apoptotic factor. Mir-18, a member of OncomiR 1 cluster, is known to target CTGF enclosing a coherent Type II FFL [Dews et al., 2006].

Coherent Type II FFLs in invasion are formed by miR-182, miR-103, and miR-124 which target the transcription factor AML. [Boominathan, 2009]. The calcium-dependent cell–cell adhesion transmembrane glycoprotein, E-cadherin is targeted by miR-183, miR-145, miR-215, miR-203, miR-205, and miR-192 in a coherent type II network (Fig. 1). Regulatory clusters involving multiple microRNA targeted genes act as a regulatory hub for invasion [Boominathan, 2009].

miR-221 and mir-222 inhibits migration, proliferation, and angiogenesis in vitro by targeting the stem cell factor receptor c-Kit through a coherent type II FFL (Fig. 2). High expression of miR-221 and miR-222 blocks angiogenesis and proliferation in endothelial cells while it promotes proliferation in cancer cells by targeting the cell cycle inhibitor p27 [leSage et al., 2007]. Due to the presence of such a characteristic regulation they may be potential therapeutic targets. miR-15, miR-16, miR-20a, and miR20b function as potential anti-angiomiRs by targeting VEGF for repression, interacting with each other to form a complex FFL [Hua et al., 2006]. MicroRNA-126 is required for vascular integrity and angiogenesis in vivo through Spred-1 and PIK3R2 constituting microRNA-TF coherent Type II FFLs [Self Fish et al., 2008]. Inhibition of IGF by miR-320 in a coherent Type II FFL is seen to improve angiogenesis [Wang et al., 2009].

The TFs Fosl2, STAT, and AML1 are regulated by multiple microRNAs and can be considered as a central hub regulating invasion and epithelial mesenchymal transition. The effectiveness of FFL is less compared to feedback loops and hence its regulation

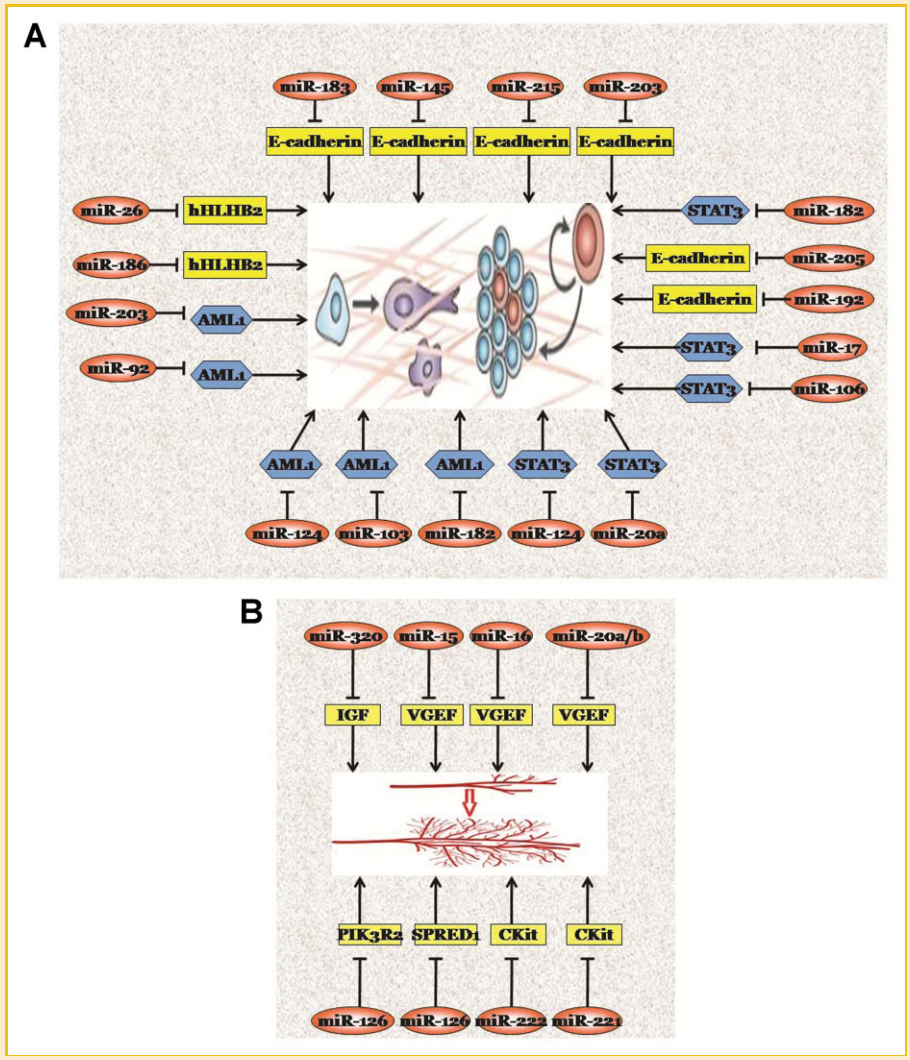

Fig. 2. A: Coherent type II FFLs in epithelial mesenchymal transition, and (B) coherent type II FFLs in angiogenesis.

requires many such loops. The presence of many coherent Type II FFLs ensures a tight control over the cell to remain in its normal state rather than to become cancerous.

INHIBITORY CONTROL TO METASTASIC EVENTS BY COHERENT TYPE IV FFLs

In coherent type IV FFL, a gene X inhibits a transcription factor which then can inhibit gene Z. The miR-17–92 cluster also named OncomiR-1 encodes miR-17, miR-18, miR-19a, miR-20a, miR-19b, and miR-92a. These promote tumor angiogenesis by targeting anti-angiogenic proteins thrombospondin-1 (Tsp1) and connective tissue growth factor (CTGF) regulating angiogenesis in a non-cell-autonomous manner [Dews et al., 2006]. Overexpression of miR-17-92 in Ras expressing cells promotes tumor angiogenesis in a coherent Type IV FFL (Fig. 3). Studies by Fasanaro et al. revealed that miR-210 enhances angiogenesis and survival response to hypoxia in vitro through Ephrin-A3 and constitutes a coherent type IV FFL [Fasanaro et al., 2008]. Antiangiogenic effects of homeobox HOXA5 are mediated by a coherent type IV FFL involving miR-130a [Yun Chen et al., 2008].

Four coherent type IV FFLs operate in invasion and epithelial mesenchymal transition processes. Zinc finger TFs, ZEB are known to regulate epithelial mesenchymal transition and its invasion. E-cadherin is a major target gene of these transcriptional repressors, and downregulation of E-cadherin is considered as a hallmark of epithelial mesenchymal transition [Boominathan, 2009]. Four microRNAs miR-183, miR-215, miR-145, and miR-192 coherently target ZEB forming Type IV FFLs. This tight regulation makes ZEB a hub protein (Fig. 3). Any mutation in ZEB can hasten the metastatic process in all types of cancers.

COHERENT TYPE I FFLs AS THE KEYS TO MIGRATORY PHENOTYPE

In coherent type I FFL, a gene X activates a transcription factor which can activate gene Z. Transition of an epithelial phenotype to a

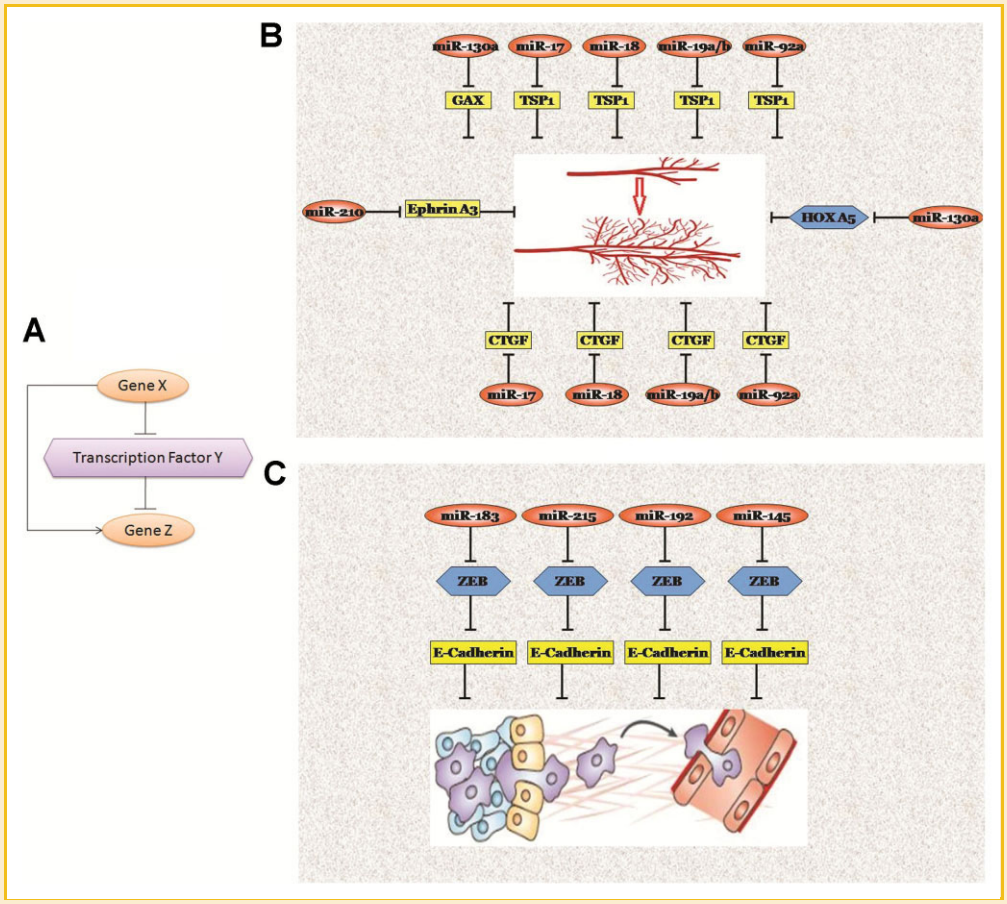

Fig. 3. A: Coherent type IV FFL, (B) coherent type IV FFL in angiogenesis, and (C) coherent type IV FFL in invasion.

mesenchymal one is activated by miR-17, miR-20a, miR-106, miR-30, miR-20b, and miR-27 through the hub transcription factor FOSL2 by an array of coherent Type I FFLs (Fig. 4). miR-155 activates the intron less gene CEBPβ in a coherent type I FFL and promotes epithelial mesenchymal transition [Boominathan, 2009].

DOUBLE POSITIVE FEEDBACK LOOP—THE MASTER SWITCH IN METASTASIS

In double positive feedback loops, two activators activate each other and have two steady states: Either both X and Y are OFF, or both are ON. A double positive loop between X and Y is useful for decisions whereby the cell irreversibly assumes a fate in response to a transient signal. Genes specific to the cell fate can be co activated by X and Y. Under normal conditions, miR-200 suppresses expression of its own repressor ZEB1, thereby favoring an epithelial phenotype and cell adhesion. In response to signals such as transforming growth factor-β, the epithelial mesenchymal transition-inducing transcription factor ZEB1 is activated and represses expression of its own suppressor miR-200 (Fig. 5), thereby favoring epithelial mesenchymal transition, invasion and probably metastasis [Vandewalle et al., 2009].

This pattern scores the most effective regulatory loop in metastasis and being regulated by the hub transcription factor ZEB1. Regulation through ZEB transcription factor is prominent in many cancers [Bracken et al., 2008]. ZEB1/2 are important regulators in the complex network of transcriptional repressors that regulate the expression of E-cadherin and epithelial mesenchymal transition through repression of multiple genes involved in transition from epithelial to mesenchymal phenotype [Vandewalle et al., 2009]. Since invasion is the first step of metastasis presence of this double positive feedback loop ensures that the cell retains its normal state.

AUTOREGULATORY LOOP—THE METASTATIC GATE KEEPER

Positive AutoRegulation (PAR) occurs when a transcription factor enhances its own rate of production. In PAR, X activates its own promoter. In positive autoregulation though the response times are slower, variation is usually enhanced [Alon, 2007].

Yannick et al. identified that E2F1, E2F2, and E2F3 directly bind to the promoter of the *mir-17–92* cluster activating its transcription, suggesting a positive autoregulatory feedback loop between E2F factors and microRNAs from the *mir-17–92* cluster [Sylvestre et al., 2007] (Fig. 6). The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target

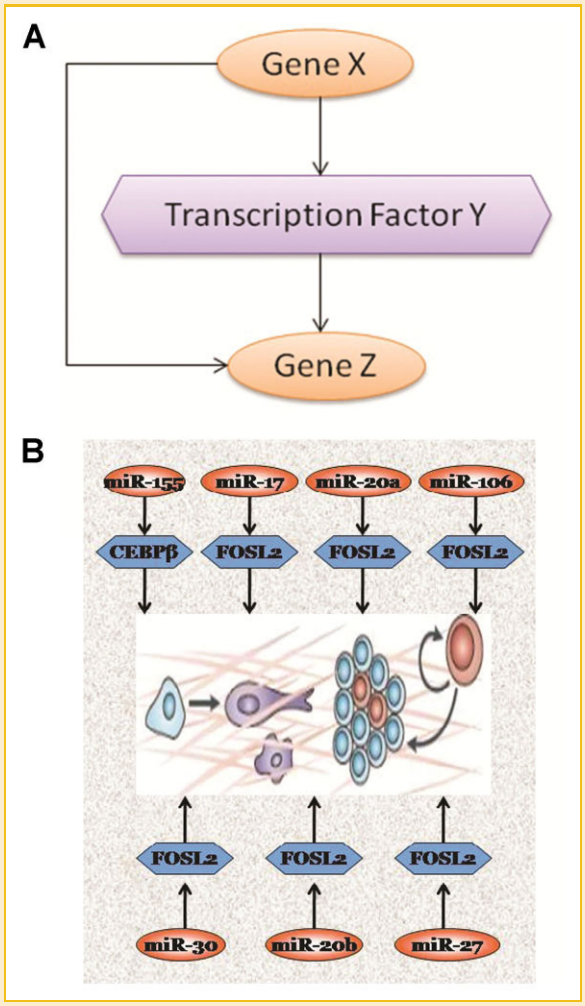

Fig. 4. A: Coherent type I FFL, and (B) coherent type II FFLs in epithelial mesenchymal transition.

of the transforming proteins of small DNA tumor viruses (Fig. 6). E2F regulation is an important regulatory mechanism as the regulation of E2F is under its own control.

Tight combinatorial regulation in metastasis usually involves a microRNA-transcription factor and the presence of a combinatorial

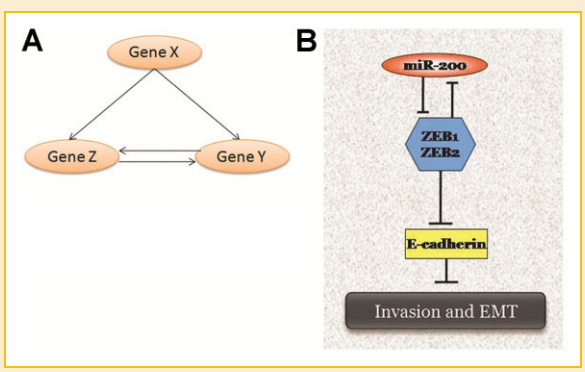

Fig. 5. A: Double positive feedback loop, and (B) double positive feedback loop in invasion and epithelial mesenchymal transition.

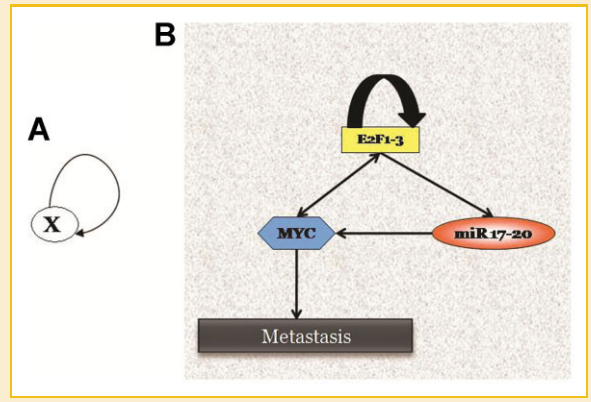

Fig. 6. A: Autoregulatory loop, and (B) autoregulatory loop in metastasis.

feedback regulatory pattern. The number of feedback loops in a network may be few but provides an immense regulatory effect. There exist a large number of coherent types II and IV FFLs. They are not subject to highly effective control unless a hub transcription factor is involved. Autoregulatory and feedback loops are crucial in the metastatic juncture and a mutation in the genes or proteins that mediate feedback loops can lead to metastatic nature of tumors. Once the impact of these loops subsides, dramatic changes begin to occur in the microenvironment leading to metastasis.

CONCLUSION AND FUTURE PERSPECTIVES

Using the available validated information, we have attempted an in silico approach to categorize the patterns of microRNAs and TFs into different regulatory modules. We further identified the regulation of ZEB1/2 genes by miR-200 family to be a core regulatory system to all cancers. We explored the possible combinatorial efforts by microRNAs and TFs to retain the cell in its normal state. Increasing evidence suggests that definite miR-TF regulatory patterns have a significant role in tumor development and prognosis. A minute alteration of pattern will provoke a chain of reaction and feedback pathways involving microRNA hubs, affecting multiple target genes of the same or different pathways. However, the role of transcriptomal patterns in tumor metastasis continues to be unclear and needs to be addressed. This may raise the curtain on elucidation of deeper mechanisms in tumor metastasis.

REFERENCES

Alon U. 2007. Network motifs: Theory and experimental approaches. Nat Rev Genet 8:450–461.

Bartel DP. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116:281–297.

Batada NN, Hurst LD, Tyers M. 2006. Evolutionary and physiological importance of hub proteins. PLoS Comput Biol 2:e88.

Bommer GT, Gerin I, Feng Y, Kaczorowski AJ, Kuick R, Love RE, Zhai Y, Giordano TJ, Qin ZS, Moore BB. 2007. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Curr Biol 17:1298–1307.

Boominathan L. 2009. p63, p73, & p53 are negative regulators of epithelial to mesenchymal transition (EMT), invasion & metastasis. Nature Proceedings DOI: 10.1038/npre. 4109.1

Boyd D, Simon C, Nemechek AJ, Boyd D, O'Malley BW Jr, Goepfert H, Flaitz CM, Hicks MJ. 1998. An orthotopic floor-of-mouth cancer model allows quantification of tumor invasion. Laryngoscope 108:1686–1691.

Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA. 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122(6): 947–956.

Bracken CP, Gregory PA, Kolesnikoff N, Bert AG, Wang J, Shannon MF, Goodall GJ. 2008. A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res 68:7846–7854.

Brosh R, Shalgi R, Liran A, Landan G, Korotayev K, Nguyen GH, Enerly E, Johnsen H, Buganim Y, Solomon H, Goldstein I, Madar S, Goldfinger N, Børresen-Dale A-L, Ginsberg D, Harris CC, Pilpel Y, Oren M, Rotter V. 2008. p53-repressed miRNAs are involved with E2F in a feed forward loop promoting proliferation. Mol Syst Biol 4:229.

Chambers AF, Groom AC, MacDonald IC. 2002. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2:563–572.

Cohen EE, Zhu H, Lingen MW, Martin LE, Kuo WL, Choi EA, Kocherginsky M, Parker JS, Chung CH, Rosner MR. 2009. A feed-forward loop involving protein kinase Calpha and microRNAs regulates tumor cell cycle. Cancer Res 69:65–74.

Deplancke B, Vermeirssen V, Arda HE, Martinez NJ, Walhout AJM. 2004. Gateway-compatible yeast one hybrid screens. Genome Res 14:2093–2101.

Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, Furth EE, Lee WM, Enders GH, Mendell JT. 2006. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet 38:1060–1065.

Farh KK, Kai-How Farh Kyle, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, Burge C, Bartel DP. 2005. The widespread impact of mammalian microRNAs on mRNA repression and evolution. Science 310:1817–1821.

Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, Capogrossi MC, Martelli F. 2008. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem 283:15878–15883.

Fazi F, Racanicchi S, Zardo G, Starnes LM, Mancini M, Travaglini L, Diverio D, Ammatuna E, Cimino G, Lo-Coco F, Grignani F, Nervi C. 2007. Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. Cancer Cell 12:457–466.

Feng R, Chen X, Yu Y, Su L, Yu B, Li JQ, Sengupta S. 2008. MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc Natl Acad Sci USA 105:5874–5878.

Harbison CT, Gordon DB, Lee TI, Rinaldi NJ, Macisaac KD, Danford TW, Hannett NM, Tagne J-B, Reynolds DB, Yoo J, Jennings EG, Zeitlinger J, Pokholok DK, Kellis M, Rolfe PA, Takusagawa KT, Lander ES, Gifford DK, Fraenkel E, Young1 RA. 2004. Transcriptional regulatory code of a eukaryotic genome. Nature 431:99–104.

Hobert O. 2008. MicroRNAs gene regulation by transcription factors and miRNAs. Science 319:1785.

Hua Z, Lv Q, Ye W, Wong CK, Cai G, Gu D, Ji Y, Zhao C, Wang J, Yang BB, et al. 2006. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS ONE 1:e116.

Hunter KW, Crawford NP, Alsarraj J. 2008. Mechanisms of metastasis. Breast Cancer Res 10(Suppl 1): S2.

Johnston RJ, Chang S, Etchberger JF, Ortiz CO, Hobert O. 2005. MicroRNAs acting in a double-negative feedback loop to control a neuronal cell fate decision. Proc Natl Acad Sci 102:12449–12454.

Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, Hannon G, Abeliovich A. 2007. A MicroRNA feedback circuit in midbrain dopamine neurons. Science 317:1220–1224.

Koshiol J. 2010. Strengths and limitations of laboratory procedures for MicroRNA detection. Cancer Epidemiol. Biomarkers Prev 19:907–911.

leSage C, Nagel, R, Egan, DA, Schrier M, Mesman E, Mangiola A, Anile C, Maira G, Mercatelli N, Ciafre SA, Farace MG, Agami R. 2007. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. Embo J 26:3699–3708.

Leber MF, Efferth T. 2009. Molecular principles of cancer invasion and metastasis. Int J Oncol 34:881–895.

Ma L, Teruya-Feldstein J, Weinberg RA. 2007. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449:682–688.

Mangan S, Zaslaver A, Alon U. 2003. The coherent feedforward loop serves as a sign sensitive delay element in transcription networks. J Mol Biol 334:197–204.

Milo R, Shen-Orr SS, Itzkovitz S, Kashtan n, Chklovskii D, Alon U. 2002. Network motifs: Simple building blocks of complex networks. Science 298(5594): 824–827.

Qihong H, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, Coukos G, Zhang L, Puré E, Agami R. 2008. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 10:2202–2210.

Sandmann T, Jensen LJ, Jakobsen JS, Karzynski MM, Eichenlaub MP, Bork P, Furlong EEM. 2006. A temporal map of transcription factor activity: Mef2 directly regulates target genes at all stages of muscle development. Dev Cell 10:797–807.

Schulte JH, Horn S, Otto T, Samans B, Heukamp LC, Eilers UC. 2008. MYCN regulates oncogenic MicroRNAs in neuroblastoma. Int J Cancer 122:699–704.

Self Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, Ivey KN, Bruneau BG, Stainier DY, Srivastava D. 2008. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 15:272–284.

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

Shen-Orr SS, Milo R, Mangan S, Alon U. 2002. Network motifs in the transcriptional regulation network of Escherichia coli. Nat Genet 31: 64–68.

Sood P, Krek A, Zavolan M, Macino G, Rajewsky N. 2006. Cell-type-specific signatures of microRNAs on target mRNA expression. Proc Natl Acad Sci USA 103:2746–2751.

Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM. 2005. Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3'UTR evolution. Cell 123:1133–1146.

Sylvestre Y, De Guire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F, Ferbeyre G, Chartrand P. 2007. An E2F/miR-20a autoregulatory feedback loop. J Biol Chem 282:2135–2143.

Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massagué J. 2008. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451:147–152.

Tsang J, Zhu J, van Oudenaarden A. 2007. MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. Mol Cell 26:753–767.

Vandewalle C, Van Roy F, Berxa G. 2009. The role of the ZEB family of transcription factors in development and disease. Cell Mol Life Sci 66:773–787.

Varghese J, Cohen SM. 2007. microRNA miR-14 acts to modulate a positive autoregulatory loop controlling steroid hormone signaling in Drosophila. Genes Dev 21:2277–2282.

Vermeirssen V, Barrasa MI, Hidalgo C, Babon JAB, Sequerra R, Doucette-Stam L, Barabasi AL, Walhout AJM. 2007. Transcription factor modularity in a gene-centered C. elegans core neuronal protein-DNA interaction network. Genome Res 17:1061–1071.

Wang E, Purisima E. 2005. Network motifs are enriched with transcription factors whose transcripts have short half-lives. Trends Genet 21:492–495.

Wang XH, Qian RZ, Zhang W, Chen SF, Jin HM, Hu RM. 2009. MicroRNA-320 expression in myocardial microvascular endothelial cell and its relationship with insulin-like growth factor-1 in type 2 diabetic rats. Clin Exp Pharmacol Physio 36:181–188.

Woods K, Thomson JM, Hammond SM. 2007. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. J Biol Chem 282:2130–2134.

Yeger-Lotem E, Sattath S, Kashtan N, Itzkovitz S. 2004. Network motifs in integrated cellular networks of transcription-regulation and protein-protein interactions. Proc Natl Acad Sci USA 101:5934–5939.

Yun Chen, Gorski DH. 2008. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. Blood 111:1217–1226.

Zhu S, Si ML, Wu H, Mo YY. 2007. MicroRNA-21 targets the tumor suppressor gene tropomyosin1 (TPM1). J Biol Chem 282(19): 14328–14336.