MicroRNA functional network in pancreatic cancer: From biology to biomarkers of disease

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MicroRNAs (miRs), the 17- to 25-nucleotide-long non-coding RNAs, regulate expression of approximately 30% of the protein-coding genes at the post-transcriptional level and have emerged as critical components of the complex functional pathway networks controlling important cellular processes, such as proliferation, development, differentiation, stress response' and apoptosis. Abnormal expression levels of miRs, regulating critical cancer-associated pathways, have been implicated to play important roles in the oncogenic processes, functioning both as oncogenes and as tumour suppressor genes. Elucidation of the genetic networks regulated by the abnormally expressing miRs in cancer cells is proving to be extremely significant in understanding the role of these miRs in the induction of malignant-transformation-associated phenotypic changes. As a result, the miRs involved in the oncogenic transformation process are being investigated as novel biomarkers of disease detection and prognosis as well as potential therapeutic targets for human cancers. In this \article, we review the existing literature in the field documenting the significance of aberrantly expressed miRs in human pancreatic cancer and discuss how the oncogenic miRs may be involved in the genetic networks regulating functional pathways deregulated in this malignancy.

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1. Introduction

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the world. With a median survival of <6 months and an average 5-year survival rate of <5%, the mortality–incidence ratio for pancreatic cancer patients is about 0.99 (Hayat *et al*. 2007). Thus, there is an urgent need to understand the molecular mechanisms underlying this disease, including the genetic networks affected during the malignant transformation process, in order to develop better detection, diagnostic and prognostic markers as well as therapeutic targets that could aid in improving clinical management and therapeutic outcome for the patients.

Recent discoveries of small non-coding microRNAs (miR) as robust regulators of gene expression that are frequently deregulated in human cancers have provided a new opportunity to unravel the aberrantly expressing cellular pathways, which underlie neoplastic transformation of cells. It has been reported that expression patterns of miRs rather than messenger RNAs (mRNAs) are more informative as pathognomonic tumourigenic events for human cancers (Lu *et al*. 2005). Genome-wide profiling

Keywords. Cellular pathways; genetic network; microRNA; pancreatic cancer; tumorigenic transformation; 3' untranslated region

Abbreviations used: 3′UTR, 3′ untranslated region; CLL, chronic lymphocytic leukemia; CP, chronic pancreatitis; IGF-IR, insulin-like growth factor type I receptor; INSR, insulin receptor; IPA, Ingenuity Pathway Analysis; IPMN, intraductal papillary neoplasms; miR, microRNAs; mRNAs, messenger RNAs; mTOR, mammalian target of rapamycin; PDAC, pancreatic ductal adenocarcinoma; PDCD4, programmed cell death 4; pre-miR, precursor miRNA; pri-miRNA, primary RNA; PTEN, phosphatase and tensin homologue 2; RISC, RNA-induced silencing complex; TIMP3, tissue inhibitor of metalloproteinase 3; TP53INP1, tumour protein p53-induced nuclear protein 1

studies from cancer cells of various organ sites have indicated that these non-coding transcripts are preferentially enriched in a tissue- and tumour-specific manner (Lu *et al*. 2005; He *et al*. 2005; Murakami *et al*. 2006; Roldo *et al*. 2006). Furthermore, functional analyses suggest that miRs play roles in cancer initiation, invasion and progression processes and, therefore, may prove to be informative biomarkers of detection, diagnosis and prognosis besides being potential targets of therapy (Esquela-Kerscher and Slack 2006; Nelson and Weiss 2008; Li *et al*. 2009).

The miRs are a class of conserved small non-coding RNAs of 17–25 nucleotides (nt) in length that regulate gene expression by either repressing the translation or causing degradation of multiple-target mRNAs (Ambros 2004; Gregory *et al*. 2005). Since the discovery in *Caenorhabditis elgans* that the *lin-4*-gene-encoded small RNAs are associated with the control of developmental timing by negatively regulating *lin-14* translation (Lee *et al*. 1993), there has been an explosion in the field of small non-coding RNA biology in subsequent years across different species. These studies have led to the current understanding that miRs have critical regulatory functions in development, proliferation, differentiation, apoptosis and stress response. Increasing number of studies in the past few years have established the regulatory roles of miRs in complex genetic networks underlying various cellular pathways.

2. Genomic organization and biogenesis of miRs

MicroRNA genes represent about 1% of the genome in different species, and it is estimated that about 30% of the protein-coding genes in the human genome are regulated by miRs (Bartel 2004). Majority of the miR genes (about 70%) are intragenic, being located in introns or exons, and the remaining (about 30%) are localized in the intergenic regions or in gene deserts as independent transcription units. The miRs may be transcribed as monocistronic (such as miR-21) or polycistronic (such as the miR-17-92-1 cluster) transcripts and regulated either under the control of the host tissue genes or independently by their own regulatory elements (Rodriguez *et al*. 2004; Zeng 2006).

Biogenesis of miRs involves several steps (figure 1). In the first step, several-kilobase-long primary RNA (pri-miRNA) is transcribed by RNA polymerase II. The pri-miR is then cleaved by an RNase III endonuclease Drosha in the nucleus into a hairpin loop structure of about 70–100 nt in length, known as the precursor miRNA (pre-miR). Pre-miRs are then transported to the cytoplasm by the RanGTP-dependent Exportin-5-mediated mechanism, where these are further processed by RNase III endonuclease, Dicer/TBP and Ago2 into 17- to 25-nt-long duplex mature miRs. Single-stranded mature miRs are then incorporated into the RNA-induced silencing complex (RISC) and direct the complex to specific mRNAs by complementary base pairing with the 3′ untranslated regions (3′UTR). In most instances the base pairing is imperfect with the 5′ half of the miR referred to as the seed region comprising of nt 2–8 pairing with the complementary sequence in the 3′UTR of the mRNAs. Partial complementarity between the seed region of the miR and the 3′UTR of the target transcript leads to inhibition of translation, while perfect complementarity results in degradation of the mRNA (Bartel 2004; He and Hannon 2004). It is estimated that, on an average, each miR may be regulating about 200 gene transcripts (Krek *et al*. 2005).

3. MicroRNAs and cancer

Aberrant expression of miRs has been detected in many human diseases including cancer (McManus 2003; Esquela-Kerscher and Slack 2006). Published studies have reported that while elevated expression of some miRs accompany carcinogenesis (oncogenes), down-regulation of others correlate with the development of cancer-associated phenotypes (tumour suppressor). The first evidence of miR involvement in cancer was obtained from studies in chronic lymphocytic leukemia (CLL) patients, where miR-15a and miR-16 were found silenced in about 69% of the patients (Calin *et al*. 2002). Interestingly, this miR cluster is localized on chromosome 13q14 locus that is frequently deleted in CLL. Following these initial observations, extensive mapping of the known miR genes revealed that these are often located in the genomic intervals rearranged in cancers including those displaying amplification, loss of heterozygosity, common breakpoints and fragile sites (Calin *et al*. 2004).

Aberrant expression of miRs has been described in almost all human cancers and experimental evidence suggests that abnormally expressing miRs have a causative role in tumourigenesis. Loss of miR-15a/16-1 in CLL, for example, reflects their tumour suppressor function with uncontrolled expression of the target anti-apoptotic gene *BCL2* in the CLL cells (Cimmino *et al*. 2005). Oncogenic function of several miRs, on the other hand, is evident since their gain of function promotes tumourigenesis following inflammation by facilitating cell proliferation over cell death. Examples include up-regulated miR-155 induced by mediators of inflammation targeting the suppressor of cytokine signalling 1 gene (Tili *et al*. 2007; Jiang *et al*. 2010) and miR-21-mediating cell survival and proliferation by targeting tumour suppressor genes *PTEN* and *PDCD4* (Meng *et al*. 2007; Asangani *et al*. 2008). With abnormal expression of miRs established as a common genetic event in cancer, an increasing number of recent investigations reveal that miR expression is regulated by the same

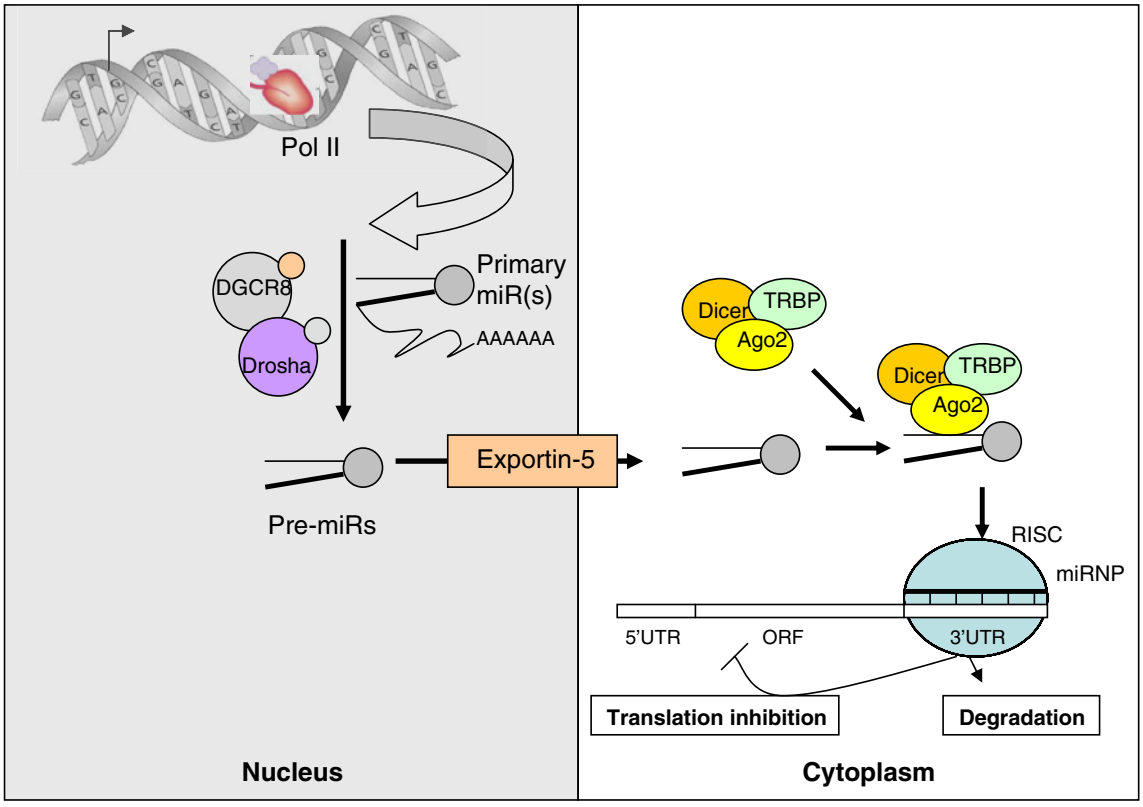

Figure 1. Overview of microRNA biosynthesis and function**.** RISC refers to the RNA-induced silencing complex.

mechanisms that control the expression of protein-coding genes including epigenetic regulation (Iorio *et al*. 2010). The abnormal expression of miRs in cancer cells can be abnormal due to defects in the miR biogenesis machinery, as evident from changes in miR expression levels consequent to altered Drosha or Dicer activity in different tumour types (Thomson *et al*. 2006). Altered miR expressions also result from changed activity of transcription factors (Chang *et al*. 2007). Additionally, epigenetic mechanisms, such as promoter DNA methylation or histone methylation and histone acetylation, modify miR expression. Aberrant epigenetic regulation of miR expression is detected in different diseases including cancer.

4. Deregulated expression of miRs in pancreatic cancer

A number of expression profiling studies have demonstrated deregulation of miR expression in pancreatic cancer tissues and cell lines (table 1). The deregulated miR expression profiles have been proposed to represent helpful markers for differential diagnosis of pancreatic cancer from chronic inflammatory disease of the pancreas and other tumours. An extensive analysis of miR expression profile in tissue samples from normal pancreas, chronic pancreatitis and pancreatic ductal adenocarcinoma (PDAC) using microarray technology revealed that some miRs, like miR-29c, miR-96, miR-143, miR-148b and miR-150, were differentially expressed in both the chronic pancreatitis and PDAC samples, whereas expression of miR-196a, miR-196b, miR-203, miR-210, miR-222, miR-216, miR-217 and miR-375 were altered only in PDAC samples (Szafranska *et al*. 2007). The authors concluded that the expression profiles of two miRs, miR-196a and miR-217, could discriminate PDAC from normal pancreas and chronic pancreatitis. In another study, elevated expression of miR-155, miR-181a, b, c, d, miR-21 and miR-221 together with down-regulation of miR-148a, b and miR-375 was reported to differentiate pancreatic cancer from pancreatitis tissue samples (Bloomston *et al*. 2007). Up-regulation of miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b and miR-95 was detected in a third study with 10 pancreatic cancer cell lines and 17 pairs of pancreatic cancer/normal tissues (Zhang *et al*. 2009). Interestingly, two of the up-regulated miRs, miR-155 and miR-21, were also found significantly elevated in the intraductal papillary neoplasms (IPMN) compared with matched controls (Habbe *et al*. 2009). Considering that IPMN are non-invasive precursor lesions of pancreatic cancer, it was concluded that aberrant expression of these miRs is an early event in the multi-stage progression of the disease.

Table 1. Deregulated microRNAs in PDAC

microRNA	Expression profile	References
Let-7f-1	Up	Lee *et al.* 2007
Let-7d	Up	Lee *et al.* 2007
miR-10	Up	Bloomston *et al.* 2007; Zhang *et al.* 2009
miR-15b	Up	Lee *et al.* 2007;Zhang *et al.* 2009
miR-16-1	Up	Lee *et al.* 2007
miR-21	Up	Lee *et al.* 2007; Bloomston *et al.* 2007; Zhang *et al.* 2009; Mees *et al.* 2010
miR-23	Up	Bloomston *et al.* 2007
miR-24	Up	Lee *et al.* 2007
miR-31	Up	Szafranska *et al.* 2007; Szafranska *et al.* 2008
miR-92	Up	Lee *et al.* 2007
miR-95	Up	Zhang *et al.* 2009
miR-96	Down	Szafranska *et al.* 2007; Szafranska *et al.* 2008
miR-99	Up	Bloomston *et al.* 2007
miR-100	Up	Lee *et al.* 2007; Bloomston *et al.* 2007
miR-103	Up	Bloomston *et al.* 2007; Zhang *et al.* 2009
miR-107	Up	Lee *et al.* 2007; Bloomston *et al.* 2007; Zhang *et al.* 2009
miR-125	Up	Lee *et al.* 2007; Bloomston *et al.* 2007
miR-130b	Down	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008
miR-139	Down	Lee *et al.* 2007
miR-142-P	Down	Lee *et al.* 2007
miR-143	Up	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008; Zhang *et al.* 2009
miR-145	Up	Szafranska *et al.* 2008; Zhang *et al.* 2009
miR-146	Up	Szafranska *et al.* 2007; Bloomston *et al.* 2007
miR-148a	Down	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008
miR-148b	Down	Szafranska *et al.* 2007; Bloomston *et al.* 2007
miR-155	Up	Lee *et al.* 2007; Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008; Zhang *et al.* 2009
miR-181a	Up	Lee *et al.* 2007; Bloomston *et al.* 2007
miR-181b	Up	Bloomston *et al.* 2007
miR-181c	Up	Lee *et al.* 2007; Bloomston *et al.* 2007
miR-181d	Up	Bloomston *et al.* 2007
miR-186	Up	Zhang *et al.* 2009
miR-190	Up	Zhang *et al.* 2009
miR-194	Up	Mees *et al.* 2010
miR-196a	Up	Szafranska *et al.* 2007; Szafranska *et al.* 2008; Zhang *et al.* 2009
miR-196b	Up	Szafranska *et al.* 2007
miR-199a	Up	Bloomston *et al.* 2007
miR-200b	Up	Zhang *et al.* 2009; Mees *et al.* 2010
miR-200c	Up	Mees *et al.* 2010
miR-205	Up	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008
miR-210	Up	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008; Zhang *et al.* 2009
miR-212	Up	Lee *et al.* 2007
miR-213	Up	Bloomston *et al.* 2007
miR-217	Down	Szafranska *et al.* 2007; Szafranska *et al.* 2008
miR-220	Up	Bloomston *et al.* 2007
miR-221	Up	Lee *et al.* 2007; Szafranska *et al.* 2007; Bloomston *et al.* 2007; Zhang *et al.* 2009; Mees *et al.* 2010
miR-222	Up	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Zhang *et al.* 2009
miR-223	Up	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008; Zhang *et al.* 2009
miR-301	Up	Lee *et al.* 2007
miR-345	Down	Lee *et al.* 2007
miR-375	Down	Szafranska *et al.* 2007; Bloomston *et al.* 2007; Szafranska *et al.* 2008
miR-376a	Up	Lee *et al.* 2007
miR-424	Up	Lee *et al.* 2007
miR-429	Up	Mees *et al.* 2010

Table 2. The validated gene targets of 11 up-regulated microRNAs in PDAC

miRNAs	Validated targets
miR-21	PDCD4, PTEN, BTG2,TGFBR2, MARCKS, pellino-1, TM1, SPRY2, TPM1, Maspin, PPARalpha, RECK, NFIB, CDC25A, LRRFIP1, BCL2, RTN4, HNRPK and TAp63
miR-210	AcvR1b, ISCU and COX10, E2F3, RAD52, ACVR1B, MNT, FGFRL1, HOXA1 and HOXA9
miR-155	TP53INP1, FOXP3, FOXO3a, CYR61, RHOA, SMAD1, SMAD5, HIVEP2, CEBPB, RUNX2, MYO10, MyD88, SHIP1, C/EBPbeta, IFN-gammaRalpha, JARID2, BACH1, ZIC3, ZNF652, ARID2, AGTR1, AT1R, RIPK1, FADD, AID, JARID2, RHOA
miR-196a	S100A9, KRT5, SPRR2C, HOXA7, HOXB8, HOXC8, HOXD8, HMGA2, ANXA1
miR-200b	RND3
miR-221	PUMA, PTEN, MDM2, PI3K, ICAM-1,p27, Bmf, Bim, p57
miR-222	PUMA, PTEN, p27, Bim, MMP1, SOD2, STAT5A,
miR-15b	Arl2, BCL2,
miR-186	P2X7
miR-190	NeuroD
miR-95	–

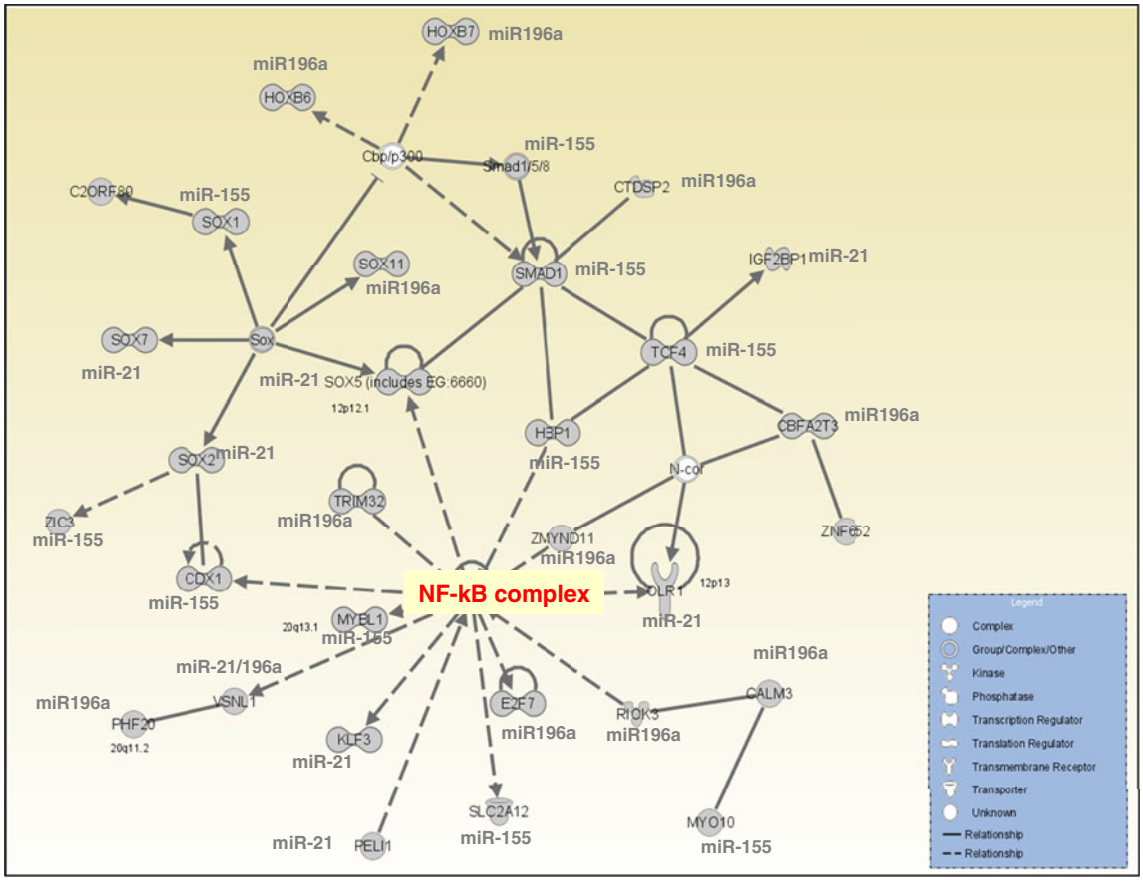

Figure 2. Network of miR-21, miR-155, miR-210 and miR-196a target genes derived with IPA. Note NF-kB as the focal node of this network.

Theoretical justification for the possible involvement of differentially expressed miRs in the malignant transformation process becomes evident from the known functions of the target genes regulated by these miRs. It is expected that multiple aberrantly expressing miRs collectively induce abnormal phenotypic changes in cancer cells by targeting the signalling pathways regulated by the genetic networks comprising of their respective target genes. Identification and functional validation of the target genes for the aberrantly expressing miRs, thus, provide important insights into the pathways affected in different tumour types. For example, miR-155 up-regulated in pancreatic cancer targets the mRNA for the stress-induced gene, tumour protein p53-induced nuclear protein 1 (TP53INP1) that is down-regulated in pancreatic cancer, expected to impair stress response in these cells. Likewise, miR-21, expressed at high levels in pancreatic cancer, negatively regulates the expression of tumour suppressor phosphatase and tensin homologue 2 (PTEN), programmed cell death 4 (PDCD4) and tissue inhibitor of metalloproteinase 3 (TIMP3) proteins, leading to inhibition of apoptosis and facilitation of invasive potential in cells acquiring progressively advanced malignant-transformation-associated phenotypes (Rachagani *et al.* 2010).

We are investigating the 11 miRs frequently over-expressed in pancreatic ductal adenocarcinomas (PDAC) regulating an array of their target genes (table 2), which displayed loss of function in the PDAC tumours and PDAC cell lines profiled in our laboratory. The miRs and the targets are being analysed for their involvement in the genetic networks and cellular pathways deregulated in this cancer. The genetic networks and cellular pathways were derived with the help of the Ingenuity Pathway Analysis (IPA) program. A total of 71 genes targeted by the 11 miRs, found to be down-regulated in our PDAC expression micro-array profiling studies, were included in the analyses performed in two separate steps. In the first step we generated a network of the genes directly targeted by the four miRs, miR-21, miR-210, miR-155 and miR-196a, which have been found in multiple independent studies to be preferentially expressed in PDAC. Interestingly, this network revealed NF-κB at a focal node reflecting an important role for this transcription factor in pancreatic carcinogenesis (figure 2), corroborating the concept that this transcription factor is essential for maintenance of pancreatic tumours through constitutive activation (Wong and Lemoine 2009). Since NF-κB is a master regulator of gene transcription during inflammation and stress response, known to trigger cellular transformation following chronic activation, it appears that the complex genetic network involving the four miRs are critical determinants of this pathway activation and therefore of their sustained induction in pancreatic cancer.

A more comprehensive network and pathway analysis of all the genes targeted by the 11 miRs revealed their association with four important network functions and five critical canonical pathways, all relevant to development of cancer-associated phenotypes. It is significant in this context that in each of the four genetic networks, the miR-targeted genes constitute about half of the total

Table 3. Genetic networks associated with the target genes of 11 microRNAs up-regulated in PDAC

Associated network functions	Score	Focus molecules	Molecules in network
Cancer, immunological disease, cell cycle	48	22	14-3-3, APLN, BACE1, BBC3, BTG2, CD4, CDKN1B, COL4A1, COL4A3, Collagen type IV, Cyclin A, ELL2, ESRRG, Foxo, FOXO1, GFAP, hCG, HOMER2, Insulin, MAP2K1/2, MYBL1, NFkB (complex), p70 S6k, p85 (pik3r), PCSK1, Pdgf, PDGF BB, PIKFYVE, PPP1R9A, PTEN, RPS6KB1, SLC4A4, TCR, TOB1, VAV3
Cell morphology, free radical scavenging, molecular transporter	27	14	ATAD2, BBC3, beta-estradiol, BOC, CDON, CRLF1, CUL4A, DCAF10, DHCR7, EID1, FOXK1,Hedgehog, hydrogen peroxide, IER5, IFI30, IFNGR2, LEPROT, MB, MIR27B, MLLT4, NDN, NHLH2, NRXN3, NXPH1, OSGIN1, PTPN3, SRSF3, STAT3, SYT3, TMCC1, TP53, TP53AIP1, TP53INP1, VAV3, YWHAB
Cardiovascular system development and function, inflammatory response, cell morphology	25	13	26 s Proteasome, Actin, ADORA2A, Akt, Caspase, Ck2, CNTFR, DMD, DTNA, DUSP1, ERK, ERK1/2, ESPN, ETV5, F Actin, FSH, GABARAPL1, GMNN, Histone h3, IgG, Immunoglobulin, INSR, Integrin, Jnk, Mapk, MLL3, P38 MAPK, PDCD4, PI3K (complex), Pka, Pkc(s), Ras, RASSF5, Tubulin, Vegf
Inflammatory disease, dermatological diseases and conditions, cancer	23	12	ABAT, ALDH5A1, Alp, ALPL, ARHGEF6/7, BRIP1, CD163, CDC42EP3, CRK, CYP4F3, DLGAP3, EPB41L4B, FIGN, GAS1, GIT2, HNF4A, IFI30, LAD1, MID1, MID1IP1, MIR124-1, MIR192, MON1B, MYB, NR3C1, PAK1, PCDH17, PECI, PGM1, PPP1R11, RBMS3, SHANK2, TGFB1, TNFAIP1, ZEB2

molecules involved and the network-associated cellular functions include those related to cancer, cell cycle, cell morphology, free radical scavenging and inflammatory response (table 3 and figure 3) that are expected to be affected during initiation and/or progression of malignancy. The genes supposedly silenced by the over-expressing miRs belong to five canonical signaling pathways, frequently deregulated in cancer (table 4). Although only five miR targets (RPS6KB1, FOXO1, INSR, CDKN1B and PTEN) with similar and overlapping signature patterns were represented in the signalling pathways, each of these five gene products have documented functional involvement in controlling cell growth and have been implicated to play roles in different human cancers. The ribosomal protein S6 kinase

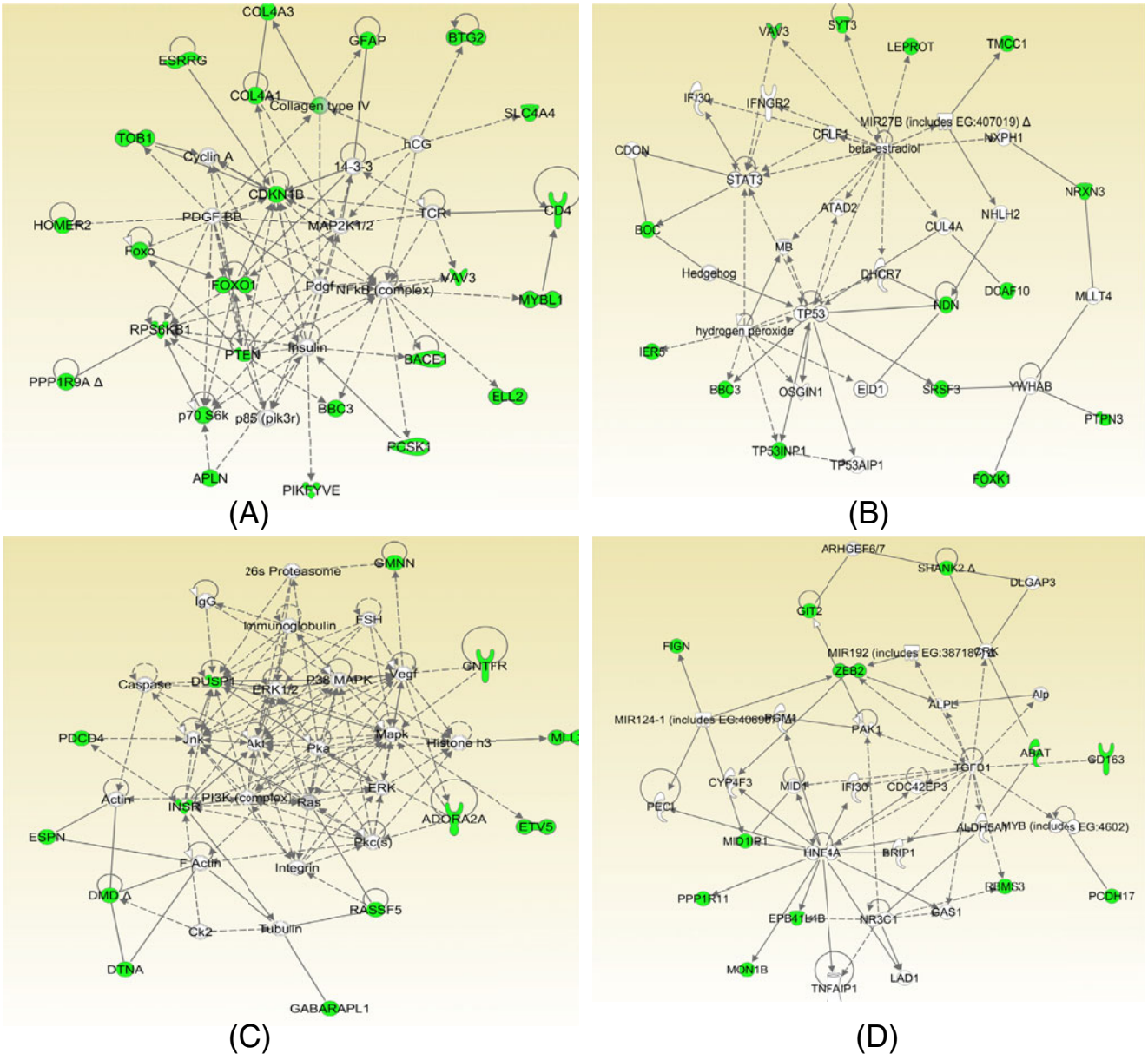

Figure 3. Four most significant networks generated by IPA consisting of microRNA targeted 71 genes deregulated in PDAC. Each node represents a gene and its association with other genes represented by a line (edge). Nodes have different shapes to represent different molecule types. The genes in networks have a colored background: Genes in green are down-regulated. Genes with no background color were inserted by IPA to generate a highly connected network. The top four functions associated with the networks are (**A**) cancer, immunological disease and cell cycle, (**B**) cell Morphology, free radical scavenging and molecular transporter, (**C**) cardiovascular system development and function, inflammatory response and cell morphology and (**D**) inflammatory disease, dermatological diseases and cancer.

Table 4. Top five signalling pathways involving genes targeted by 11 up-regulated microRNAs in PDAC derived with IPA

Signalling pathway	*P*-value	Ratio	Molecules
PTEN signalling	8.48E−05	5/123 (0.041)	RPS6KB1,FOXO1,INSR,CDKN1B,PTEN
Insulin receptor signalling	2.20E−04	5/141 (0.035)	RPS6KB1,FOXO1,PPP1R11,INSR,PTEN
PI3K/AKT signalling	1.66E−03	4/142 (0.028)	RPS6KB1,FOXO1,CDKN1B,PTEN
Glioblastoma multiforme signalling	3.45E−03	4/166 (0.024)	RPS6KB1,FOXO1,CDKN1B,PTEN
Prostate cancer signalling	5.27E−03	3/100 (0.030)	FOXO1,CDKN1B,PTEN

family member, RPS6KB1, function is important for cell growth following activation by growth factors such as EGF, PDGF and insulin regulated by mammalian target of rapamycin (mTOR). The FOXO transcription factors regulate several biological processes such as stress resistance, metabolism, cell cycle, apoptosis and DNA repair. It has been suggested that FOXO transcription factors act in a negative feedback regulatory manner with the pro-survival PI3K/AKT and Erk signalling pathways, activated in cancer cells. The role of insulin receptor (INSR) in regulating growth and metabolism has also been characterized and reported to be active as hybrid heteromers with insulin-like growth factor type I receptor (IGF-IR) in tumour cells. The CDKN1B/p27 is a member of the Cip/Kip family of cyclin-dependent kinase inhibitors, which functions as tumour suppressor protein due to its ability to induce cell cycle arrest and is found inactivated in multiple tumour types. Finally, PTEN tumour suppressor, although commonly deregulated in pancreatic cancer, is mutated at a very low frequency and its targeting by miRs provides a mechanistic explanation of frequent silencing in pancreatic cancer. Studies with targeted deletion of PTEN in mouse models have indicated that dysregulation of PTEN/PI3K/AKT and Ras/Raf pathways act synergistically to promote pancreatic cancer initiation and progression (Hill *et al.* 2010).

In addition to the miRs frequently up-regulated in PDAC, several miRs have been found to be down-regulated in pancreatic and other cancers, as well. We have, however, primarily focused on the biology of up-regulated miRs simply because the presence rather than the absence of a marker is expected to give more confidence in developing a biomarker assay for disease detection and prognosis. Nonetheless, a few of the down-regulated miRs have been investigated in detail in terms of their biology and thus potential roles as tumour suppressors. For example, miR-34a down-regulated in PDAC is directly trans-activated by tumour suppressor protein p53. Expression of miR-34a induces reprogramming of gene expression affecting cell cycle progression, DNA repair, angiogenesis and promotes apoptosis. Transcriptional silencing of miR-34a due to promoter CpG methylation has been demonstrated in cancer cells (Roldo *et al*. 2006). Significant down-regulation of miR-let-7 in PDAC with complete loss of expression in poorly differentiated tumours is another example of miR inactivation that has been characterized for underlying biology in the tumour cells. The fact that let-7 negatively regulates expression of K-Ras and activation of MAPK indicates that loss of expression of this miR is expected to promote cancer cell proliferation (Moriyama *et al*. 2009). In view of the differentially expressing oncogenic and tumour suppressor miRs targeting multiple genes in important cancer-relevant genetic networks and cellular signalling pathways in pancreatic cancer, it is logical to suggest that distinct miR expression signatures may be associated with different grades and stages of this malignancy.

5. MicroRNAs as biomarkers of pancreatic cancer

The robust regulatory role of each miR in controlling expression of multiple gene transcripts offer a unique opportunity of developing miR signatures as informative biomarkers for detection, diagnosis and prognosis of tumours that result from deregulation of complex genetic networks. This underlying biological principle most likely was the reason why expression patterns of 217 miRs were found to classify majority of tumour types more accurately than the information based on expression profile of ~16000 mRNAs (Lu *et al*. 2005).

As mentioned above, previous studies have identified miR signatures specific for normal pancreas, chronic pancreatitis (CP) and cancer tissues. These findings revealed that presence of miR-216 and miR-217 and absence of miR-133a is a characteristic of pancreatic tissue and that a total of 26 miRs are aberrantly expressed in PDAC. Among these, only down-regulation of miR-217 and elevated expression of miR-196a can discriminate normal pancreas and CP from PDAC. Additionally, eight differentially expressed miRs were reported to differentiate pancreatic cancer from CP (Szafranska *et al*. 2007). Another study suggested that endocrine tumours of the pancreas could be distinguished from acinar type by a set of 10 miRs, which possibly associated with normal endocrine differentiation or endocrine tumourigenesis (Roldo *et al*. 2006), indicating the diagnostic utility of miR expression signatures in pancreatic

cancer. We have recently demonstrated that differentially expressing miRs in PDAC can also be profiled in blood as a minimally invasive biomarker assay for pancreatic cancer (Wang *et al*. 2009). This finding is extremely promising since there are no reliable biomarkers assays, much less of minimally invasive nature, currently available for early detection, diagnosis and predicting prognosis of pancreatic cancer patients. A number of studies have, of late, suggested prognostic significance of miR expression profiles in pancreatic carcinomas. For example, a subgroup of six miRs was reported to distinguish long-term survivors with node-positive disease from those succumbing within 24 months and elevated expression of miR-196a-2 was predictive of median survival differing by about a year among pancreatic cancer patients (Bloomston *et al*. 2007). Furthermore, up-regulation of miR-155, miR-203, miR-210 and miR-222 was also found to be significantly associated with poorer survival of patients with pancreatic carcinomas (Greither *et al*. 2010).

6. Therapeutic targeting of miRs in pancreatic cancer

In view of the functional involvement of miRs in the deregulation of genetic networks underlying the development and progression of pancreatic cancer, attempts are underway to develop therapeutic strategies against cancer-associated miRs for patients with this malignancy. Targeting the mature miRs or their precursors with synthetic chemically modified anti-sense oligonucleotides to silence the over-expressing transcripts or rescuing the expression levels of those down-regulated in cancer cells are being investigated for therapeutic purposes. These investigations appear promising based on the following results obtained so far. Inhibition of over-expressing miR-21 and miR-221 in pancreatic cancer cell lines results in cell cycle arrest accompanied by increased expression of tumour suppressor PTEN, RECK and p27 proteins. The miR antisense oligonucleotides in combination with the commonly used therapeutic drug gemcitabine had a synergistic effect in impeding the growth of pancreatic cancer cells (Park *et al*. 2009). Restoration of let-7 expression level in PDAC cells was found to inhibit proliferation although failed to inhibit tumour growth progression *in vivo* after intra-tumoural gene transfer as a single agent (Torrisani *et al*. 2009). More promising results were, however, obtained following rescue of miR-34 expression level in pancreatic cancer cells, which led to inhibition of clonogenic cell growth, invasion and induction of apoptosis accompanying down-regulation of Bcl-2 and Notch pathways. These cells were also more sensitive to chemotherapy and radiation with 87% reduction in tumour-initiating cell population together with significant inhibition of tumour sphere growth *in vitro* and tumour formation *in vivo* (Ji *et al*. 2009). The findings provide compelling reasons to pursue the targeting miRs as a novel molecular therapeutic approach for pancreatic cancer.

7. Conclusions

The significant functional involvement of miRs in tumour development and progression has been fairly well established and justifies further in-depth investigations to characterize their complex regulatory roles in human cancers. Future studies should include discovery projects to identify novel miRs in the tissues and corresponding tumours together with characterization of their detailed biological functions under different physiological states. Unraveling the role of miRs in various genetic networks and regulatory pathways responsible for the maintenance of normal cellular homeostasis as well as development of transformed phenotypes would allow better utilization of these molecules as biomarkers of human cancers. Successful therapeutic targeting of miRs also holds significant promise towards improved clinical management of patients with cancer especially those with pancreatic carcinomas since these patients have very limited treatment options available at this time

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