Placental microRNAs as potential regulators in tumour processes

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ABSTRACT
Trophoblasts and cancerous cells share a number of commonalities, undertaking processes such as cell proliferation, angiogenesis, tissue invasion, evasion of apoptosis and immune control, among others that have been described as hallmarks of cancer. The Trophoblastic Thesis of Cancer sustains that despite the similarities between placental and tumour cells, trophoblasts face meticulous regulation. Epigenetic regulation by specific placental miRNAs has been described, through computational biology methods, the complete miRNA quantification Transcriptome Profiles from breast, cervix, ovary and prostate cancer available in TCGA project were compared with placental normal tissue to find overexpressed placental miRNAs. Differential expression analysis showed the C19MC miRNAs as the highly expressed miRNAs, functional annotation of these miRNAs revealed the MAPK signaling pathway as the greatly regulated pathway in placenta with a total of 45 miRNAs regulating 135 genes. MAPK signaling pathways are commonly dysregulated in tumours but carefully regulated in embryonic development, demonstrating that C19MC miRNA epigenetic regulation has a potential role in tumour progression control and cancer prognosis.

KEYWORDS Trophoblastic Thesis of Cancer, C19MC, MAPK, hallmarks of cancer, epigenetic regulation.

INTRODUCTION

Both cancer and an early embryo face the same problematic scenario: being a tissue with high nutritional demands and high rate of development, both committed to host colonization. The similarities between the cells that originate the placenta and promote the implantation of the embryo, the trophoblasts, and the malignant cells that develop into cancer include the presence of undifferentiated cells, angiogenesis, tissue invasion, cell proliferation, resistance to cell adhesion, evasion of immune control and apoptosis avoidance, processes included in the hallmarks of cancer (Meirson et al. 2020).

From the end of the 19th century, Scottish embryologist John Beard proposed the Trophoblastic Theory of Cancer, based on choriocarcinoma observations, a type of cancer in the placenta which is one of the most aggressive gestational trophoblastic neoplasms (Jun et al. 2020). According to Beard, cancer develops when trophoblast invasion escapes regulation by day 56 of human embryonic development. By then, Beard proposed that cancer cells came from cells he identified as dormant trophoblasts (today known as stem cells) that migrated throughout the body during embryonic development and restarted trophoblastic processes (Ross 2015). Beard is currently considered one of the forerunners of cancer theory of stem cell division, according to which changes in the genetic material required for carcinogenesis develop in stem cells or end up in stem cells following dedifferentiation processes; based in the idea that cancer progression requires cells with the ability to transmit their mutations and self-renew attributes (Moss 2008; López-Lázaro 2018).

Nevertheless, Trophoblastic Theory of Cancer sustains that cancer does not originate from dedifferentiated somatic cells but originates from stem cells that managed to escape normal regulatory control, consequently reactivating trophoblastic characteristics (Ross 2015). Shared molecular signals between trophoblasts and cancer cells that trigger cellular processes have been identified, including the expression of proto-oncogenes (eg c-myc, c-ras, c-erbB1), growth factors (eg EGF, IGF-2), endopeptidase enzymes of the matrixin family, hormones (eg prolactin, metastin, HCG, CRF, GH), and tumour-associated antigens (eg hCG-β, OPN) (Ferretti et al. 2006).

It has been proposed that the transformation of benign tumour cells into cancer could be a consequence of the acquisition of trophoblastic properties while continuing to maintain the characteristics of the tissue of origin. This perspective allows the understanding of why cancer types diversity is often dependent on the tissue from which the tumour cells arise (Doello 2018). Embryonic development and precancerous to cancerous cell transition necessarily involve changes in the gene expression profile. These expression changes are mainly regulated by epigenetic mechanisms, such as DNA methylation, histone modification, protein-coding RNAs (mRNAs), and non-coding RNAs (ncRNAs) such as lincRNA, circRNA, snoRNA, piRNA, siRNA, and miRNA (Vincent & van Seuningen 2012). The characteristics of microRNAs (miRNAs) have positioned them as biomarkers for cancer diagnosis, prognosis and treatment, for example, the silencing of miR-137 has been associated with tumour proliferation and angiogenesis in prostate cancer, while miR-137 expression acts as a tumour suppressor (Peng & Croce 2016; Guan et al. 2019).

MicroRNAs are small non-coding RNAs conformed by 19-24 nucleotides which target the 3 'untranslated regions (3'-UTR) of nearly 50% of human mRNA transcripts. These ncRNAs are involved in translational suppression and transcriptional silencing triggering chromatin remodelling (Filipów & Łaczmański 2020). It has been widely reported that the aberrant expression of miRNAs is involved in carcinogenesis, exhibiting simultaneously high tissue specificity, consequently allowing the classification of different cancer types based on their expression profile (Pichler & Calin 2015). However, little has been studied of shared cellular processes epigenetically regulated by miRNAs between embryonic development and tumour progression.

Researches conducted from an epigenetic perspective have identified more than 2,500 human miRNAs, of which more than 700 miRNAs are found in the placenta. Several of these placental miRNAs are trophoblast specific, such as the C19MC (chromosome 19 microRNA cluster), located in chromosome 19q13.41 in humans, this cluster is expressed almost exclusively in embryonic stem cells and the placenta, encoding 59 mature miRNAs (Liu et al. 2018; Bullerdiek & Flor, 2012; Zhao et al. 2018). Additionally, studies have determined the association between certain miRNAs and cancer hallmarks; it has been shown that these ncRNAs act as oncogenes or as tumour suppressors under differential microenvironments (Pichler & Calin 2015). Aberrant expression of miRNAs in cancer arises from the amplification or deletion of miRNAs, transcriptional control errors, transcription factor dysregulation, abnormal epigenetic changes, and defects in the biogenesis machinery of miRNAs (Peng & Croce 2016).

The placenta is a tissue highly dependent on hormones, molecules which modulate embryo implantation, placentation, vascular remodelling and immunomodulation, as well as the development of each gestational event (Costa 2016). Carrying out a miRNA differential expression analysis in placenta and other tissues under high hormonal load with presence of malignant tumours would allow the finding of miRNAs that participate in the regulation and control of embryonic development but have differential expression under a tumour

progression scenario and normal tissue. There are striking phenotypic similarities between trophoblast and cancer with respect to the ability to invade the host organism and migrate in the organism. The concept of trophoblastic type transdifferentiation of cancer cells can be useful in identifying those molecular mechanisms that provide an explanation of how key dysregulation processes have occurred in cancer cells, where trophoblastic properties are vital, in contrast to cells in which these properties are permanently silenced epigenetically (Piechowski 2019). In such a way, identifying those miRNAs that are specifically found in the placenta, will allow us to propose miRNAs as regulators of metabolic pathways and genes of trophoblastic processes, which vary in similar processes in cancer cells.

MATERIALS AND METHODS

Data obtention

Raw counts of miRNAs from five Placental Tissues were obtained from the GEO database (ncbi.nlm.nih.gov/geo). Sample accession IDs taken were GSM1901233, GSM1901234, GSM1901235, GSM1901236 and GSM1901237 from the series GSE73713. The tissue compartment from which these samples were taken was villous trophoblast from individuals with a mean gestational age of 39.1 weeks. For Uterus, Breast, Cervical and Prostate, Normal and Cancerous Tissue samples (Table 1), raw counts of miRNAs were obtained from the GDC database (portal.gdc.cancer.gov). Also, the complete miRNA quantification Transcriptome Profiles available for these types of cancer were obtained from The Cancer Genome Atlas Project (TCGA): TCGA-BRCA (1207 files from 1079 cases), TCGA-PRAD (551 files from 494 cases), TCGA-CESC (312 files from 307 cases), and for TCGA-UCEC (579 files from 550 cases).

Table 1. Normal and Cancer tissues samples UUIDs.

Sample	UUID
Uterus sarcoma	7dc24dec-1d71-4a7e-8cbe-58ce4b6eeb47
Breast cancer	3157a8cb-4f6b-4a7a-a3b5-d4868302a295
Cervical carcinoma	968a657a-1fe8-4027-be7a-4c076c29f8a61
Prostate cancer	04c0fae1-849e-4fc4-ad35-edc6d7b7f44d1
Uterus normal tissue	3a95f223-8916-4468-8a88-af3b0aa15ce6
Breast normal tissue	2db0a3fd-2399-4bfb-b676-2b19c197f391
Cervical normal tissue	52e8a415-ea02-499b-a663-dbb82b8fd7b5
Prostate normal tissue	ddab496d-35d6-45c0-b82d-e60498ff95f0

Data Normalization and Differential Expression Analysis

Making use of R version 4.0.2 and Bioconductor project version 3.11, RNA-seq analysis was performed using limma, Glimma and EdgeR packages (Law et al. 2016). Considering miRNAs counts as an input, and putting them through pre-processing and exploratory data analysis before obtaining lists of differentially expressed (DE) miRNAs. DE microRNAs were divided into Up- or Down-regulated miRNAs, a P-value < 0.05 and a fold-change ≥ 5 were set as the cut-off values of DE miRNAs. For TCGA data analysis DESeq2, GenomicDataCommons and TCGAbiolinks packages were used for Normalization and Differential Expression Analysis (Colaprico et al. 2015).

Functional annotation of DE miRNAs

For obtaining the functional annotation based on enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways of DE microRNAs, the tool DIANA-miRPath v3.0 was used (Vlachos et al. 2015), which employs TargetScan (www.targetscan.org), microT-CDS (www.microrna.gr/microT-CDS) and TarBase v7.0 (www.microrna.gr/tarbase) databases for experimentally supported interactions for DE miRNAs.

RESULTS

After performing the differential expression analysis of Placenta vs Tumour samples, 78 Up-regulated and 25 Down-regulated miRNAs were found considering a fold-change above 5 and under -5 respectively, these results showed data homogeneity for both comparisons (Figure 1). As part of the 78 differentially expressed (DE) miRNAs, those from the C19MC have shown to be the most Up-regulated placental miRNAs compared to cancerous tissues (Figure 2).

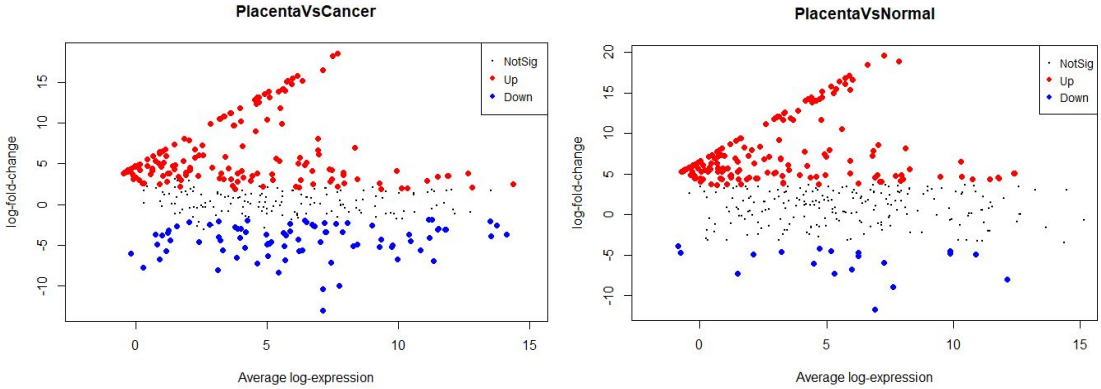

Figure 1. (Left) Differentially Expressed miRNAs between Placenta and Cancerous Tissues, (Right) and between Placenta and Normal Tissues.

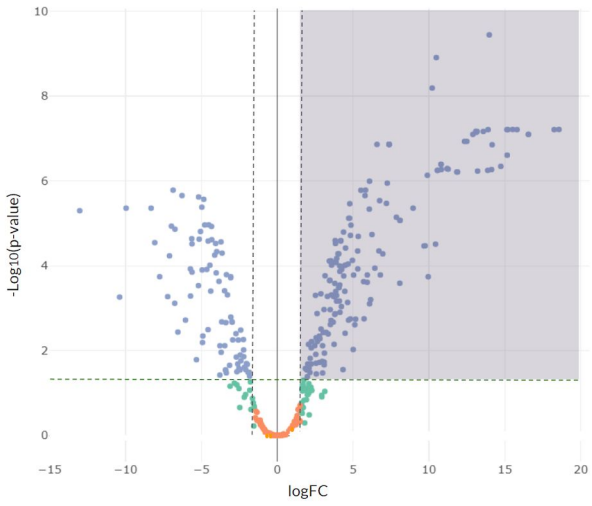

Figure 2. Volcano plot for DE miRNAs between Placenta and Cancerous Tissues. The vertical line at zero divides up- and down-regulated miRNAs, horizontal line sets significantly DE miRNAs above the fold change value.

Placental and TCGA data for breast (BRCA), prostate (PRAD), cervical (CERC) and uterine (UCEC) cancer, DE analysis showed 78 shared up-regulated miRNAs and 15 down-regulated miRNAs in placental samples when compared with the four types of cancer considered (Figure 3). Functional annotation of C19MC miRNAs exhibited a high gene regulation in the placenta for both embryonic and tumour progression key KEGG metabolic pathways (Table 2). Suggesting a meticulous epigenetic regulation by miRNAs of these pathways in Placenta but not in the evaluated cancerous tissues.

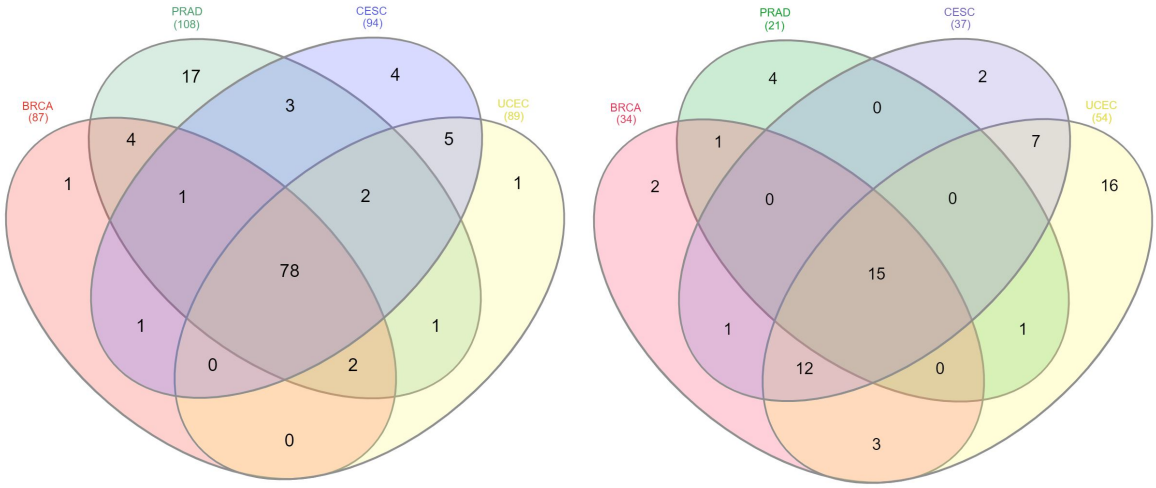

Figure 3. Venn Diagram of (Left) Up-regulated and (Right) Down-regulated DE miRNAs between Placenta and TCGA samples. In red: TCGA-BRCA; green: TCGA-PRAD; purple: TCGA-CESC; yellow: TCGA-UCEC.

Table 2. KEGG pathways for up-regulated placental (C19MC) miRNAs, considered pathways have p > 0,05 as a threshold value of significance.

Metabolic pathways	**Number of miRNAs**	**Number of Genes**	**P-value**
MAPK signaling pathway	45	135	7,68E-05
FoxO signaling pathway	37	76	1,32E-02
Wnt signaling pathway	36	76	1,32E-02
AMPK signaling pathway	34	73	1,00E-05
Adherens junction	33	45	1,78E-07
ErbB signaling pathway	33	57	6,09E-05
TGF-beta signaling pathway	31	50	3,49E-08
Ras signaling pathway	31	95	1,47E-02
mTOR signaling pathway	30	36	3,06E-02
TNF signaling pathway	28	58	7,38E-03
p53 signaling pathway	25	36	9,79E-03

DISCUSSION

Some miRNAs have been identified almost with exclusive expression in placental tissue, such as the C19MC gene cluster, suggesting a key role of miRNAs in fetal development and regulation of trophoblast behaviour (Donker et al. 2012; Thamotharan et al. 2017). Decreased expression of these ncRNAs has been associated with processes related to the Hallmarks of Cancer such as angiogenesis, tumour invasion, cell proliferation, cell migration, immune evasion and apoptosis resistance.

The Trophoblastic Thesis of Cancer sustains in the similarities found between tumour cells and trophoblasts, the constituent cells of the placenta. Despite sharing biological processes and pathways, placental and cancer tissues differ in the presence of regulatory molecular mechanisms (Ross, 2015; Vidal et al. 2018)

In this research, 78 miRNAs were found as highly expressed in placental tissue compared to the four types of cancer tissues evaluated. From these miRNAs, 45 are described as part of the C19MC out a total 52 miRNAs reported as a part of this cluster. Upregulation of the C19MC miRNAs is associated with apoptosis, regulation of stem cells pluripotency, adherens junction, and MAPK, FoxO, Wnt, AMPK, ErbB, TGF-beta, Ras, mTor, TNF and p53 signaling pathways.

The mitogen-activated protein kinase (MAPK) signaling pathway resulted as the pathway regulated by the greatest number of miRNAs. MAPK pathways have been reported as a mechanism which prevents apoptosis and promotes cellular proliferation by regulating a series of protein kinase cascades (Pérez-Pérez et al. 2008).

In humans, MAP kinases are grouped in three main families: ERKs (extracellular signal-regulated kinases) which induces cell growth, proliferation and differentiation, JNKs (Jun kinases) mainly associated with apoptosis, inflammation, cytokine production and metabolism, and p38/SAPKs (stress-activated protein kinases) which activation is involved with cell cycle regulation, cell differentiation, senescence and apoptosis (Morrison 2012; Sun et al. 2015).

Also, MAPK pathway is intimately associated with trophoblast invasion during normal pregnancies, and as a trigger for the MEK/ERK signaling cascade, a pathway associated with the regulation of gene expression, cellular proliferation, differentiation, angiogenesis, embryo development and tumour invasion (Anton et al. 2012). ERK/MAPK signaling cascade has also been found as crucial for the proper growth, differentiation and morphogenesis of the placenta (Nadeau & Charron, 2014).

The MAPK pathway is frequently activated in human cancers, leading to malignant phenotypes such as autonomous cellular proliferation. DNA-damaging agents reduce glucose transporter 3 expression in cancer cells through activation of the MEK/ERK pathway independently of p53, leading to cell death or apoptosis in HeLa cells (Sun et al. 2015). Aberrant activation events of the ERK/MAPK signaling pathway is considered a hallmark of cancer, product of hyperactivation of genes such as RAS and BRAF, which trigger a cascade of phosphorylation of downstream kinases (Cotto-Rios et al. 2020).

A total of 135 genes involved in the MAPK signaling pathway expression were found regulated by 45 overexpressed up-regulated placental miRNAs of the C19MC. These genes are classified in some of the key

gene families associated with embryonic development and cancer progression, such as CACN, SOS, RAS, BRAF, JNK, IL1R, TRAF, TAB, NFKβ, ELK, AT2, MAPK, DUSP and FGF family genes (Figure 4).

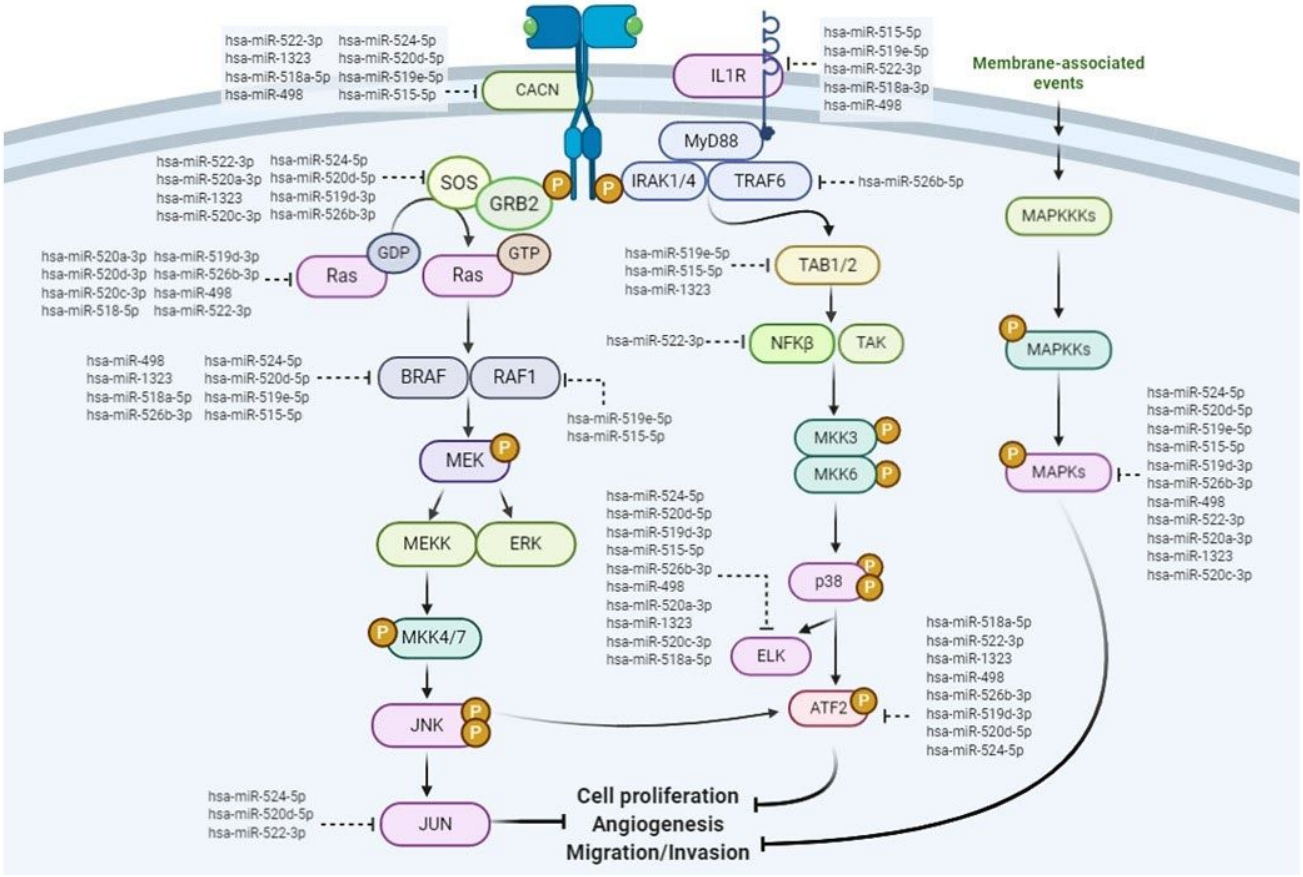

Figure 4. Regulation of placental C19MC miRNAs over genes associated with the MAPK signaling pathway, resulting in inhibition of cell proliferation, angiogenesis and migration/invasion.

Son of Sevenless (SOS) is a RasGEF receptor with a critical role for Ras activation and allosteric modulation (Christensen et al. 2016). RAS signaling pathway mediates cellular processes such as differentiation, apoptosis, cell proliferation and cell survival. RAS/RAF signaling induces c-Myc protein expression, which binds to DNA segments involved in transcriptional control of gene expression and cell proliferation (Zhang & Liu 2002). RAS gene family (KRAS, NRAS and HRAS) mutations are common genetic drivers in several types of cancers. Mutations in RAS disrupt the guanine exchange cycle by becoming independent of GAP (GTPase activating proteins) and maintaining RAS active in a GTP-bound state, triggering downstream signaling pathways which promote tumour cell growth and carcinogenesis (Moore et al. 2020; Zenonos & Kyprianou 2013).

The BRAF serine/threonine kinase is a critical effector of the ERK/MAPK signaling pathways (Cotto-Rios et al. 2020). Oncogenic mutations of the BRAF gene can be generated by gene fusion, chromosomal rearrangements and single-nucleotide variations or insertions/deletions that result in BRAF proteins with altered kinase activity (Weinberg et al. 2020). BRAF mutations, like a substitution of glutamic acid for valine

in codon 600 in exon 15 (V600E), are one of the most frequent genetic aberrations found in tumour tissues such as ovarian carcinomas. These mutations promote MAPK activation through phosphorylation resulting in dysregulation in cancer cells proliferation and survival (Sadlecki et al. 2017).

JUN N-terminal kinase (JNK) signaling pathways, often activated by environmental stress, growth factors and inflammatory cytokine stimuli, are found deregulated in cancer. JNKs are associated with transformations in oncogene and growth factor-mediated pathways. JNKs can transduce signals for differentiation in the hematopoietic cells, also, its hyperactivation is involved in embryonic development (Zhang & Liu 2002). Other tumorigenesis related functions controlled by JNKs are survival and migration of cells (Wagner & Nebreda 2009).

IL1R (interleukin-1 receptor) signaling processes are achieved through the recruitment of molecules such as MyD88, TRIF and TRAM, resulting in the activation of IRAKs (interleukin-1 receptor-associated kinases), those kinases with a C-terminal domain are eventually required for TRAF-6 activation (Rhyasen & Starczynowski 2015). IRAKs are essential mediators of inflammatory responses and are usually active in multiple types of cancer (Hosseini et al. 2018).

TAK-1 binding proteins (TAB1-3) form an oligomeric complex with TABs. TAB/TAK activation is involved in homeostasis maintenance and tumour suppression but has also been found associated with hallmark traits of cancer such as immune evasion, metastatic dissemination and microenvironment inflammation through ROS and NOS accumulation under dysregulated conditions (Mukhopadhyay & Lee 2020)

Nuclear factor-kappa B (NF-κB) is a family of transcription which regulate cell proliferation, survival, differentiation and regulate diverse immune processes. NF-κB factors are frequently activated in many types of cancer and have been found to mediate tumoural cells survival and anti-apoptotic function by preventing the activation of the pro-apoptotic protein kinase JNK (Vacarezza & Vitale 2016).

Activating transcription factor 2 (ATF2) activation is regulated by JNK and p38 signaling pathways (Kirsch et al. 2020). ATF2 contributes to DNA damage response, stress response, development and cell growth. Partial deregulation of ATF2 has been associated as a cooperator in cancer progression, affecting tumour suppressor or oncogene activities (Lopez-Bergami et al. 2010).

Dual-specificity phosphatases (DUSPs) family is regulated through gene transcription, protein modification and epigenetic modifications such as miRNA-mediated gene silencing (Huang & Tan 2012). DUSPs are critical for MAPK, ERK, JNK and p38 activity regulation, inactivation of these pathways is mediated by DUSP dephosphorylation activity of their threonine/serine and tyrosine residues (Chen et al. 2019).

Fibroblast growth factors (FGFs) signaling pathways play key roles in development, metabolism and tissue homeostasis, as well as in the regulation of a wide range of biological processes, including cell proliferation, migration, survival and differentiation (Turner & Grose 2010). FGFs malfunction or mutations promote tumour growth, angiogenesis, invasion and metastasis, for example, FGF5 is overexpressed in breast cancer, FGF16 in ovarian tumours and FGF23 promotes the progression of prostate cancer (Xie et al. 2020).

Through cross-communication, MAPK pathways are carefully regulated by other signaling pathways (Zhang & Liu 2002). Other pathways regulated by highly expressed C19MC placental miRNAs are FoxO, Wnt, AMPK, ErbB, TGF-beta, Ras, mTOR, TNF and p53 signaling pathways, which have shown to be involved in cancerogenesis and tumour progression (Yang et al. 2020).

Furthermore, changes in miRNA expression profiles have been described for several cancers, including breast cancer, leukaemia, prostate cancer, among others. Therefore, miRNAs may regulate major signaling networks and molecular mechanisms necessary for the development of the human placenta, carcinogenesis and tumour progression, such as proliferation, migration, invasion, and apoptosis (Onofre et al. 2018). Consistent with the importance of these findings, which provide evidence of dysregulation of epigenetic mechanisms in cancer for processes both cancer progression and embryo development, these miRNAs, particularly C19MC placental miRNAs provide new perspectives for further research, both in vivo and in vitro, for novel therapeutics design, tumour progression control, as well as improved individual characterization and prognosis for cancer patients.

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ANNEXES

Annex 1. C19MC miRNAs which regulate genes associated with the MAPK signaling pathway. (In green: miRNAs used for Gene Interactions)

miRNAs	Genes	miRNAs	Genes	miRNAs	Genes
hsa-miR-520d-5p	42	hsa-miR-519c-3p	19	hsa-miR-520g-3p	3
hsa-miR-524-5p	42	hsa-miR-519a-3p	18	hsa-miR-520h	3
hsa-miR-519e-5p	31	hsa-miR-520f-3p	15	hsa-miR-520f-5p	2
hsa-miR-515-5p	30	hsa-miR-512-3p	14	hsa-miR-516b-5p	2
hsa-miR-519d-3p	29	hsa-miR-519d-5p	13	hsa-miR-519a-5p	1
hsa-miR-526b-3p	27	hsa-miR-518c-5p	10	hsa-miR-518b	1
hsa-miR-498	25	hsa-miR-525-5p	9	hsa-miR-518d-3p	1
hsa-miR-522-3p	23	hsa-miR-516a-3p	9	hsa-miR-518a-3p	1
hsa-miR-520a-3p	22	hsa-miR-526b-5p	9	hsa-miR-518c-3p	1
hsa-miR-520d-3p	22	hsa-miR-520a-5p	8	hsa-miR-523-5p	1
hsa-miR-1323	22	hsa-miR-512-5p	5	hsa-miR-519b-5p	1
hsa-miR-520c-3p	21	hsa-miR-515-3p	4	hsa-miR-519c-5p	1
hsa-miR-518a-5p	20	hsa-miR-519e-3p	4	hsa-miR-526a	1
hsa-miR-519b-3p	19	hsa-miR-1283	3	hsa-miR-518d-5p	1

Annex 2. Total Up-regulated placental miRNAs compared with tumoural tissues.

miRNA	logCPM	logFC	Adj P Value	miRNA	logCPM	logFC	Adj P Value
hsa-mir-516b-1	7.669	18.56	6,14E-08	hsa-mir-524	2.849	9.89	7,37E-07
hsa-mir-516b-2	7.5	18.25	6,19E-08	hsa-mir-525	3.746	9.744	3,38E-05
hsa-mir-517a	7.125	16.55	8,00E-08	hsa-mir-518f	3.709	9.67	3,41E-05
hsa-mir-517b	7.125	16.55	8,00E-08	hsa-mir-7-2	4.567	8.95	4,39E-06
hsa-mir-522	6.143	15.8	6,19E-08	hsa-mir-521-1	1.849	8.086	8,51E-06
hsa-mir-1323	5.976	15.51	6,14E-08	hsa-mir-521-2	1.849	8.086	8,51E-06
hsa-mir-520g	5.791	15.2	6,19E-08	hsa-mir-495	6.943	8.074	2,58E-04
hsa-mir-520a	6.345	15.15	2,47E-07	hsa-mir-520f	2.037	7.85	7.183
hsa-mir-516a-2	5.743	15.13	6,19E-08	hsa-mir-941-1	1.464	7.368	1.38e-7
hsa-mir-516a-1	5.92	14.73	4,54E-07	hsa-mir-941-2	1.464	7.368	1.38e-7
hsa-mir-519d	5.606	14.16	1,41E-07	hsa-mir-941-3	1.464	7.368	1.38e-7
hsa-mir-518b	5.589	14.12	5,39E-07	hsa-mir-941-4	1.464	7.368	1.38e-7
hsa-mir-519a-1	5.69	13.97	3.62e-10	hsa-mir-941-5	1.464	7.368	1.38e-7
hsa-mir-523	5.067	13.89	6,19E-08	hsa-mir-934	2.542	7.247	1.127
hsa-mir-518e	5.447	13.87	5,65E-07	hsa-mir-433	4.071	7.193	3.386
hsa-mir-519c	4.891	13.58	6,83E-08	hsa-mir-224	8.334	6.954	5.229
hsa-mir-519b	5.077	13.2	5,86E-07	hsa-mir-3690-2	1.13	6.792	1,64E-04
hsa-mir-519a-2	4.667	13.17	6,83E-08	hsa-mir-329-1	2.255	6.744	2.916
hsa-mir-517c	4.634	13.11	7,19E-08	hsa-mir-493	6.941	6.698	4.471
hsa-mir-512-1	4.625	13.09	6,83E-08	hsa-mir-1278	1.024	6.571	1.38e-7
hsa-mir-512-2	4.625	13.09	6,83E-08	hsa-mir-519e	9.114	6.429	1,14E-04
hsa-mir-1283-2	4.515	12.89	8,00E-08	hsa-mir-7704	9.093	6.242	1.836
hsa-mir-1283-1	4.692	12.49	1,17E-07	hsa-mir-370	6.949	6.153	6,29E-04
hsa-mir-518c	4.6	12.34	1,17E-07	hsa-mir-548o-2	2.408	06.08	7,87E-04
hsa-mir-520h	3.953	11.88	6,15E-07	hsa-mir-548o	2.408	06.08	1.016
hsa-mir-526b	5.491	11.83	6,15E-07	hsa-mir-329-2	2.558	6.064	4.623
hsa-mir-520d	3.615	11.26	5,27E-07	hsa-mir-520b	6.626	5.932	1,64E-04
hsa-mir-518a-1	3.581	11.2	5,15E-07	hsa-mir-371a	1.151	5.925	2.449
hsa-mir-518a-2	3.581	11.2	5,15E-07	hsa-mir-494	3.388	5.814	1.651
hsa-mir-520c	3.367	10.81	5,39E-07	hsa-mir-1185-1	2.239	5.784	2.216
hsa-mir-515-1	3.346	10.79	4,07E-07	hsa-mir-1-1	6.234	5.717	1.775
hsa-mir-515-2	3.346	10.79	4,07E-07	hsa-mir-450a-2	5.353	5.691	2.363
hsa-mir-518d	3.226	10.56	5,65E-07	hsa-mir-320c-2	4.481	5.513	1.664
hsa-mir-526a-1	3.226	10.56	5,65E-07	hsa-mir-3158-1	7.465	5.336	203

hsa-mir-526a-2	3.226	10.56	5,65E-07	hsa-mir-450a-1	5.449	5.306	1.184
hsa-mir-498	3.18	10.48	1.24e-9	hsa-mir-3690-1	1.794	5.298	4.489
hsa-mir-372	4.981	10.43	309	hsa-mir-1-2	6.497	5.162	1.808
hsa-mir-7-3	4.005	10.21	6.49e-9	hsa-mir-373	1.069	5.108	2.452
hsa-mir-543	5.553	9.945	1,81E-04	hsa-mir-3158-2	8.824	05.03	1.653

Annex 3. Total Down-regulated placental miRNAs compared with tumoural tissues.

miRNA	logCPM	logFC	Adj P Value	miRNA	logCPM	logFC	Adj P Value
hsa-mir-10a	7.107	-13.02	5.039	hsa-mir-210	5.013	-6.278	2.216
hsa-mir-375	7.133	-10.39	5.457	hsa-mir-1976	-0.19	-6.049	1.908
hsa-let-7a-1	7.732	-9.963	4,39E-06	hsa-mir-33a	1.167	-5.723	1.186
hsa-let-7e	5.436	-8.316	4,39E-06	hsa-mir-374a	6.221	-5.716	5,16E-04
hsa-mir-708	3.137	-8.076	2.847	hsa-mir-374b	3.31	-5.637	23
hsa-mir-153-2	2.662	-7.751	1.809	hsa-mir-196b	6.351	-5.619	3.047
hsa-mir-338	4.631	-7.221	5,34E-04	hsa-let-7b	10.84	-5.618	1.412
hsa-mir-29c	7.438	-7.103	5.834	hsa-mir-200b	4.157	-5.328	1.632
hsa-mir-10b	11.33	-6.975	1.163	hsa-mir-183	9.258	-5.196	294
hsa-mir-379	5.674	-6.865	1.651	hsa-mir-93	9.751	-5.183	2.389
hsa-mir-2355	8.989	-6.758	7.643	hsa-mir-142	8.267	-5.148	2.358
hsa-mir-182	9.972	-6.741	1.372	hsa-mir-425	5.651	-5.061	1.557
hsa-mir-200a	3.826	-6.551	365				