BBA - Reviews on Cancer 1872 (2019) 188306

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review

From squamous intraepithelial lesions to cervical cancer: Circulating microRNAs as potential biomarkers in cervical carcinogenesis

Fernanda Costa Brandão Berti[a], Amanda Salviano-Silva[a], Helen Cristina Beckert[a], Karen Brajão de Oliveira[b], Gabriel Adelman Cipolla[a], Danielle Malheiros[a,*]

[a] Laboratory of Human Molecular Genetics, Department of Genetics, Federal University of Paraná, Av. Cel. Francisco H. dos Santos, 100, Zip Code 81530-000 Curitiba, PR, Brazil

[b] Laboratory of Molecular Genetics and Immunology, Department of Pathological Sciences, State University of Londrina, Rod. Celso Garcia Cid PR 445, KM 380, Zip Code 86057-970 Londrina, PR, Brazil

ARTICLE INFO

Keywords:
HPV
Circulating miRNAs
Cell-free miRNAs
EV-derived miRNAs
Squamous intraepithelial lesions
Cervical cancer

ABSTRACT

Despite the essential role of Human Papillomavirus (HPV) in cervical carcinogenesis, other factors are required for cancer establishment, like miRNAs. Such molecules present a complex biogenesis, being diversely distributed across tissues and biological fluids, as cell-free miRNAs or miRNAs present in extracellular vesicles (EV). After HPV infection, an interplay between HPV and the miRNA network occurs in cervical cells. As the virus persists and cellular transformation occurs, specific patterns of miRNA expression are found in different stages of cervical disease. Thus, defining promising miRNAs/specific miRNA signatures - especially circulating miRNAs - represents an interesting strategy for screening (diagnosis, prognosis, etc.) those stages. Despite the limited number of studies investigating circulating miRNAs in distinct biological fluids, accumulating data have pointed to some promising candidates, both as cell-free or EV-derived miRNAs. Here we highlight some of these promising non-invasive biomarkers and bring attention to the urgent need for efforts in this field.

1. Introduction

Cervical cancer (CC) is one of the commonest cancers in women worldwide, with approximately 500,000 new diagnosed cases every year [1]. In developing countries, especially due to inadequate healthcare services and limited resources, CC is the main cause of death by cancer among women [2]. Cervical carcinogenesis is closely related with Human Papillomavirus (HPV), an oncogenic virus actively involved in cervical epithelium transformation. After HPV infection and persistency, squamous intraepithelial lesion (SIL) development may occur, which may heal or persist and evolve to cancer. CC is mainly considered a consequence of a long-term HPV infection (although some cases evolve in a short period of time), basically resulting in the incorporation of the viral DNA into the host DNA, overexpression of oncogenic genes (such as *E6* and *E7*) and induction of oncogenic transformation. Deregulation of cell cycle and disturbance of apoptotic pathways (as well as the induction of chromosomal instability) are processes essentially involved in cervical carcinogenesis [3].

Despite overwhelming evidence that certain subtypes of HPV are the main causative agents of SIL development and progression to CC, it is also well-established that HPV alone is not sufficient to induce cervical malignant transformation. Other significant cofactors contribute to the multi-step process of cervical carcinogenesis, mainly individual genetic variations and environmental factors [4]. At the cervical microenvironment, several components appear to play important roles in HPV infection establishment, persistency, and progression to SIL and CC. Among these components, we highlight the role of microRNAs (miRNAs) - endogenous, noncoding RNA molecules of approximately

Abbreviations: CC, cervical cancer; HPV, Human Papillomavirus; SIL, squamous intraepithelial lesion; miRNAs, microRNAs; Ago, Argonaute protein; EVs, extracellular vesicles; LR- HPV, low-risk Human Papillomavirus; UR- HPV, undetermined-risk Human Papillomavirus; HR-HPV, high-risk Human Papillomavirus; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; pri-miRNA, primary miRNA; pre-miRNA, precursor miRNA; RISC, RNA-induced silencing complex; UTR, untranslated region; oncomiRs, oncogenic microRNAs; RB, retinoblastoma protein; hTERT, human telomerase reverse transcriptase; Upa, urokinase-type plasminogen activator; LAMB3, Laminin 5 β3; HFK, human foreskin keratinocytes; MAPK, mitogen-activated protein kinase; MV, microvesicles; AB, apoptotic bodies; MVB, multivesicular bodies; ESCRTs, endosomal sorting complexes required for transport; ISEV, International Society for Extracellular Vesicles; SCC, cervical squamous cell carcinoma; LNM, lymph node metastasis; SCC-Ag, SCC antigen

* Corresponding author.
E-mail address: dani_malheiros@ufpr.br (D. Malheiros).

https://doi.org/10.1016/j.bbcan.2019.08.001
Received 28 March 2019; Accepted 1 August 2019
Available online 06 August 2019
0304-419X/

22 nt in length, presenting a remarkable ability to posttranscriptionally modulate gene expression by binding to complementary sequences in the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs) [5,6].

Accumulating data have shown that miRNA profiles are surprisingly informative, reflecting the developmental lineage and differentiation stages of distinct tumors, being able to successfully categorize human cancers [7,8]. The underlying molecular mechanisms of miRNAs in cancer are not yet fully understood, but specific patterns of miRNA expression have been associated with many tumor types, including CC. Several miRNAs have been detected as differentially expressed in distinct degrees of SIL and stages of CC, as has been demonstrated their ability to be functionally involved in signaling pathways directly associated with cervical carcinogenesis [9]. Such knowledge is becoming quite solid for cellular miRNAs, although an ongoing effort still needs to be maintained, in order to properly define a reliable miRNA cellular signature in SIL and CC.

Besides being present in cellular compartments, miRNAs are also present in biofluids, such as blood, saliva and urine, occurring under both physiological and pathological conditions [10]. Generally known as circulating miRNAs, these transcripts can be associated with proteins, especially with Argonaute (Ago) protein, or packed into extracellular vesicles (EVs). In the present review, we considered two types of circulating miRNAs: those that are associated with Ago protein and defined as cell-free miRNAs (or as non-EV-derived miRNAs); and those that are packed into EVs, defined as EV-derived miRNAs.

In contrast to cellular miRNAs, little is known about cell-free and EV-derived miRNAs in different degrees of SIL and CC. Thus, the aim of this review is to provide an updated perspective about circulating miRNAs, comprising data about cell-free and EV-derived miRNAs from SIL to CC, identifying circulating miRNAs (or specific miRNA signatures) as potential biomarkers of cervical carcinogenesis stages. Additionally, despite our focus on circulating miRNAs, we also present a brief overview about cellular miRNAs in different stages of cervical carcinogenesis, in order to establish connections (or reveal the gaps) between what is found within cellular and extracellular compartments.

2. HPV and cervical carcinogenesis

In the cervical microenvironment, during a certain point of the woman's life, a physiological process characterized by the replacement of the glandular epithelium by the squamous epithelium occurs, leading to the establishment of a large area of immature squamous metaplastic epithelium – defined as the transformation zone. This region is characterized by the presence of transforming cells, and it is the commonest site of the cervix for abnormal cells to develop, being a quite vulnerable area for HPV infection [11].

As mentioned before, several factors are involved in cervical carcinogenesis, with HPV infection being a strictly necessary event for this outcome. According to its carcinogenic potential, HPVs are classified as low-risk (LR), undetermined-risk (UR) or high-risk (HR) HPVs, with the latter being involved in cervical carcinogenesis. Among HR-HPV, HPV-16 and HPV-18 stand as the most important for SIL and CC development. HPV-16 is the most predominant type, present in > 50% of CC cases annually diagnosed. Together with HPV-18, they respond for the great majority of CC cases [12–14].

In spite of the carcinogenic potential of HR-HPVs, < 5% of infected individuals develop CC [15]. This low percentage of individuals progressing to CC is highly associated with an efficient host clearance of the virus, which is observed in almost all individuals with a competent immune response. On the other hand, when immune competence is somehow impaired and/or other factors take action, abnormal changes within the host cells continuously occur, increasing the risk of SIL and, consequently, of CC development [16].

Progression to CC from a pre-invasive state to an invasive cancer itself is a slow process, taking years/decades to occur. Generally, after HPV infection and persistency, an abnormal growth of squamous cells on the surface of the cervix may be observed, characterizing SIL stages. According to the extension of the affected tissue and disposal of abnormal cells, SIL can be classified as low-grade (LSIL) or high-grade (HSIL) squamous intraepithelial lesions. In LSIL, approximately one third of the cervical epithelium is compromised, presenting a productive HPV infection, characterized by intense viral replication and mild cellular alterations. HSIL, on the other hand, compromises more than two thirds of the cervical epithelium, leading to cell cycle progressive deregulation, increased cellular proliferation and decreased cellular maturation and viral replication rates [17,18]. After LSIL establishment and persistence, progression to HSIL may occur, and, as HSIL persists, an important event may take place – the integration of HPV genome into the host genome. Viral integration is an imperative step for cervical carcinogenesis and may lead to disruption of an essential transcriptional repressor gene (*E2*), increasing the expression of *E6* and *E7* oncogenes [3]. As HSIL progresses, a transformed and irreversible phenotype may be established, culminating in CC development [19].

Despite the tight interplay between oncogenic HPV and CC, and the well-established understanding that HPV causes virtually all cases of CC, it is clearly known that factors other than HPV infection are also involved and actively participate in cervical transformation, initially in SIL development and/or later in CC progression. Indeed, CC onset tends to be the result of combined factors [20]. Genetic and epigenetic factors are crucial elements in such context, with miRNAs standing as important players [21,22].

3. MiRNAs: general features and carcinogenic potential

Microribonucleic acids, or simply miRNAs, are ~22 nt RNA molecules with broad phylogenetic distribution and posttranscriptional gene expression regulation function. The human genome fathers at least 2600 mature miRNA sequences, derived from over 1900 precursor molecules [23]. By binding to mRNA in a base-pairing manner, miRNAs are thought to regulate one third of the human transcriptome, meaning these molecules potentially participate in the regulation of every biological event. Furthermore, such widespread role is explained by features related to miRNA genomics, maturation and function. These molecules may originate from intergenic or intronic regions, i.e., may be expressed by the activity of their own promoter or depend on the expression of their host gene, respectively. Nevertheless, transcription resulting in the long primary miRNA (pri-miRNA) is carried out by RNA polymerase II. Still in the cell nucleus, this pri-miRNA is then processed by an RNase III known as Drosha, giving rise to one (solo miRNA gene) or more (clustered miRNA gene) precursor miRNAs (pre-miRNA), characterized by a stem-loop, single stranded RNA molecule lacking the long pri-miRNA overhangs. Through Exportin 5 (EXP5) and RAN-GTP, the ~60–70 nt pre-miRNA is translocated from the nucleus to the cytoplasm, where it is further processed by another RNase III, Dicer. This second processing event gives rise to a short, double stranded RNA molecule known as the miRNA:miRNA* duplex. The star symbol is used to indicate the less abundant miRNA species derived from the duplex. More recently, however, specialists have been using 5p and 3p suffixes to refer to the miRNAs that originate from the 5′ and 3′ arms of the pre-miRNA, respectively, as miRNA abundancy varies among cell and tissue types. Finally, one miRNA molecule derived from the duplex is loaded into the RNA-induced silencing complex (RISC), formed as well by the Ago protein. In this state, the miRNA will guide posttranscriptional repression by binding, at its 5′ end known as the seed sequence (2–8 nt), to the 3′ untranslated region (UTR) of the target mRNA. Such base-pairing will allow for mRNA degradation or translational repression [6,24–26].

To the previously described canonical landscape, several unusual routes have been added. Among these, we can mention: antisense or exonic genomic origin; non-conventional Drosha processing; non-conventional Dicer processing; importation of mature miRNAs to the

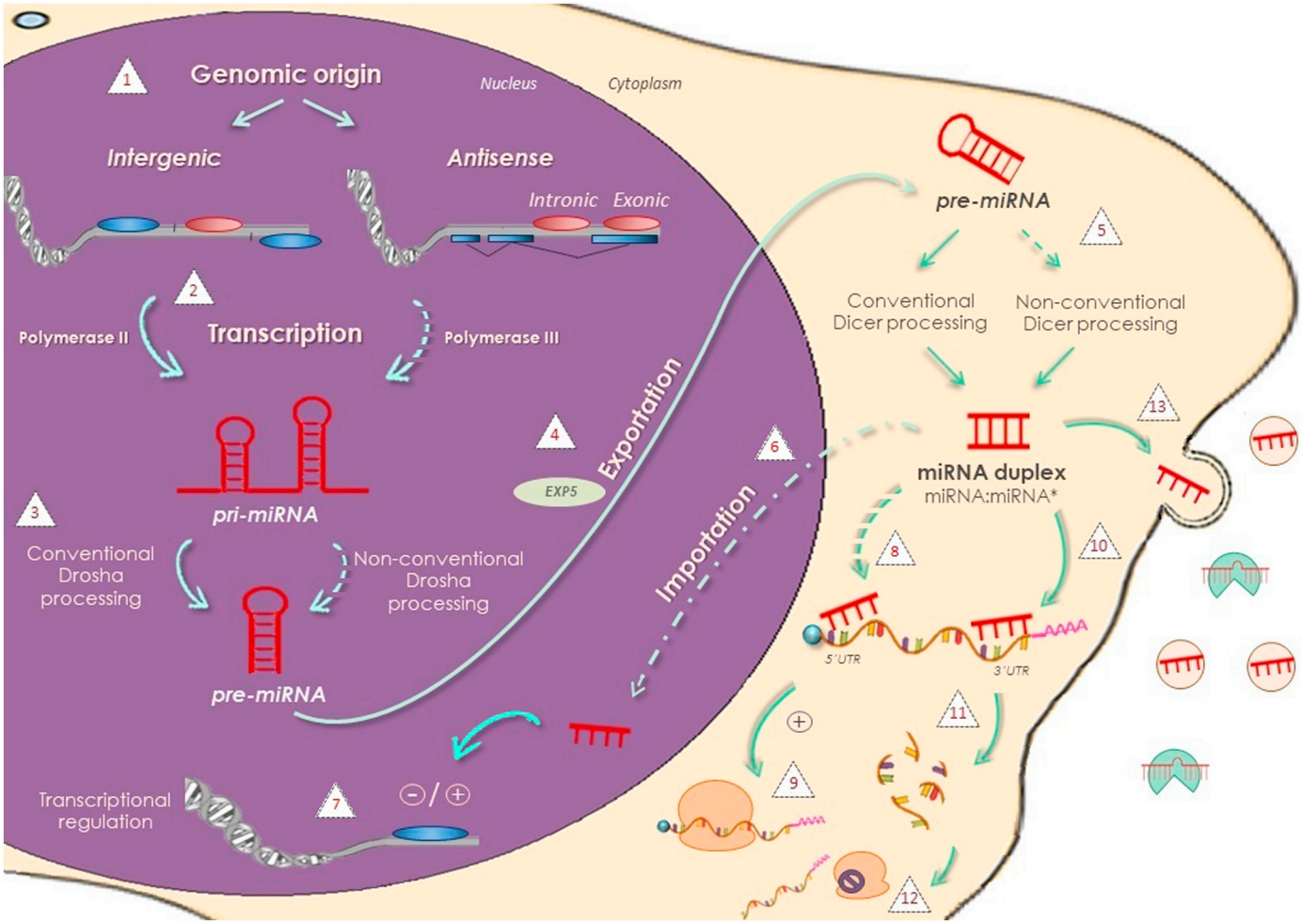

Fig. 1. Schematic overview of the miRNA biosynthesis system: the canonical (full arrows) and non-canonical (dashed arrows) pathways. Conventionally, miRNA genes reside in intergenic regions carrying their own promoters or in intronic regions of transcription units, therefore sharing the host gene promoter (1), being commonly transcribed by RNA polymerase II (2). Occasionally, miRNA *loci* also give rise to miRNAs from antisense strand transcription and processing (1), being optionally transcribed by RNA polymerase III (2). The pri-miRNA are commonly processed by Drosha into pre-miRNA, in a conventional Drosha processing, although a non-conventional Drosha processing is still possible (3). The pre-miRNA is now exported from the nucleus to the cytoplasm by EXP5 (4). Once in the cytoplasm, the pre-miRNA is generally converted into a miRNA duplex (miRNA:miRNA*) by a conventional Dicer processing, and occasionally by a non-conventional Dicer processing (5). From the miRNA duplex, one strand is loaded into the RISC complex. Non-conventionally, the importation of mature miRNAs to the nucleus may occur (6), where they may guide positive or negative transcriptional regulation (7). Optionally, miRNAs may remain in the cytoplasm and target the 5′UTR of an mRNA (8), exerting positive (as facilitating the translation process) (9) or negative posttranscriptional regulation. Commonly, the miRNA strand loaded into RISC may target the 3′UTR of the mRNA (10), leading (in a sequence-specific manner) to mRNA degradation (11) or translation inhibition (12). Finally, miRNAs may be secreted to the extracellular environment, where they may bind to carrier proteins as Ago or loaded within EVs (13). Ago, Argonaute protein; EXP5, Exportin 5; EVs, Extracellular Vesicles; RISC, RNA-induced silencing complex; UTR, untranslated region. (Image source: Mind the Graph platform; https://mindthegraph.com).

nucleus, where they may guide RNA activation or transcriptional silencing; targeting of 5′ UTR or coding regions; and positive post-transcriptional regulation [27]. Together, canonical and non-canonical landscapes reflect the complexity and importance of the miRNA system, as summarized in Fig. 1.

In addition to their complex biogenesis, miRNAs are diversely distributed across tissues and biological fluids. In other words, besides present in the cellular compartment, miRNAs may also be found extracellularly. Secretion of cellular miRNAs to the extracellular environment, where they may circulate within EVs or bound to carrier proteins (see section 5), was initially thought to be an unusual event. However, it is now broadly reported that secreted miRNAs have important physiological roles in homeostasis maintenance. In fact, there is considerable evidence that miRNAs shuttled within exosomes, a type of EV, may bind to cell receptors, further inducing changes in the recipient cell's physiology. By acting as hormones, the exosomal miRNA content may play an important role, for example, in the crosstalk between the tumor microenvironment and cancer cells [28]. Moreover, miRNA expression is known to be established by transcriptional and post-transcriptional regulatory events, which may be influenced by endogenous and exogenous factors [29]. Hence, another explanation for miRNA widespread importance would be the plasticity of miRNA expression, resulting in singular expression profiles under virtually every different set of stimuli.

4. HPV versus cellular miRNAs: impacts on cervical carcinogenesis

Several human miRNAs are known to be involved in carcinogenesis. Many of these miRNAs either contribute to or repress the cancer phenotype by inhibiting the expression of tumor suppressors or oncogenes, respectively. In cancer genetics, miRNAs have been classically categorized as either oncogenic or tumor suppressive, with oncogenic miRNAs (oncomiRs) being overexpressed and tumor suppressive miRNAs being generally underexpressed. Interestingly, a specific miRNA can act as either an oncomiR or a tumor suppressor, depending

on the context. A reasonable explanation for such contradiction relies on the fact that a particular miRNA molecule has the capacity to target different mRNAs, some of which may have opposing oncogenic or tumor suppressive functions [30].

Concerning cervical carcinogenesis, HPV seems to exert an important effect over the cellular miRNA network. Most miRNAs involved in this process seem to be expressed as a result of HPV infection, leading to critical genomic alterations. Additionally, certain miRNAs originated from the HPV genome (including the validated HPV-16-miR-H1-1 and HPV-16-miR-H2-1) appear to target crucial host genes, such as genes related to cell cycle progression, migration and immunological response, being essential for viral infection and maintenance [31]. Despite HPV low viral load and technical challenges (facts that for now turn difficult the study of these viral transcripts and their use for clinical utility), the investigation of the impact of HPV-derived miRNAs on the human genome might help us to understand viral pathogenic mechanisms. In contrast, HPV oncoproteins, especially E6 and E7, have been extensively studied and are well described as important viral molecules with a strong impact on the human genome, regulating the expression of several genes, including cellular miRNAs and their targets [32].

The miRNA processing proteins Dicer and Drosha are dysregulated in many types of tumors. In CC, the expression of these proteins is higher in HPV-positive cell lines, in comparison with HPV-negative ones, suggesting an alteration of miRNA expression by E6 and E7, possibly contributing to cervical carcinogenesis [33]. Drosha was found upregulated in all HPV CC cell lines, while Dicer was only increased in HPV-16-positive lineages. After transfection of the viral proteins E6 and E7, a significant increase of both proteins was also observed in HPV-negative CC cell lines. Moreover, many miRNAs processed by Drosha were dysregulated in E6/E7-expressing cells, especially the miRNAs involved in cell cycle, migration, development, growth and proliferation pathways [34].

HR-HPV oncoproteins E6 and E7 are closely related to cervical transformation, through a great diversity of mechanisms [3]. Some of them are widely known, such as E6 targeting the tumor suppressor p53 for proteosomal degradation (affecting many p53 transcriptional targets), and E7 inducing the retinoblastoma protein (RB) degradation, releasing the transcriptional factor E2F. Other mechanisms have been recently described, such as E7 indirectly interacting with the proto-oncogene *c-Myc* through E2F activation, regulating many miRNAs (some of them actually with binding sites in the mRNA of E2F), or E6 interacting directly with *c-Myc* [35,36]. HPV-E6 and E7 seem to interact with *c-Myc*, inducing its binding to *hTERT* (human telomerase reverse transcriptase) gene promoter, consequently promoting the reverse transcription of the HPV genome [37,38]. Thus, E6 and E7 co-expression may lead to an enhancement of this process.

As a consequence of E6-mediated reduction of p53 in CC cells, the following may be observed: downregulation of the tumor suppressor miR-34a (in HPV-16-positive and HPV-18-positive cells), inducing cell proliferation and altering important cell cycle regulators [39]; downregulation of miR-139-3p, leading to cell proliferation and migration [40]; upregulation of the oncomiR miR-21 through repression of the tumor suppressor *PTEN*, promoting cell proliferation and migration; downregulation of miR-27a and of the tumor suppressor miR-218 [41]; downregulation of miR-23b by p53 suppression (or epigenetically due to *DNMT1* upregulation), leading to the proto-oncogene *c-MET* activation, which in turn may prevent apoptosis, and increase the urokinase-type plasminogen activator (uPA), known as a metastasis promoter [42,43].

In a p53-independent manner, E6 downregulates miR-218 in HPV-16 cancer cells, as well as its tumor suppressor host gene *SLIT2*. The reduction of miR-218 upregulates Laminin 5 β3 (LAMB3), an overexpressed protein in CC cells which was described as a direct target of miR-218 [44,45]. Furthermore, miR-9, which is strongly regulated by E6 (and less by E7), was the most associated miRNA with HPV infection *status* in a transcriptome analysis, and was also shown to induce cell migration [46]. Moreover, E6 upregulates miR-20a and miR-20b [47,48]. Increased miR-20a promotes cell proliferation through induction of AKT/p38 pathway, and down-regulates the mir-20a target PDCD6, a protein known for its pro-apoptotic and growth-inhibitory effects [48]. The overexpressed miR-20b negatively regulates the metastasis suppressor *TIMP-2*, promoting epithelial-mesenchymal transition, migration and invasion of CC cells [47]. Furthermore, E6 and E7 strongly suppress miR-424 levels, while *CHK1* (a miR-424 target involved in damage repair) is increased in HPV-16 and HPV-31-positive cells. When miR-424 is overexpressed, CHK1 levels decrease, suppressing HPV genome amplification [49].

As for E6, E7 may modulate distinct miRNA expression rates. In human foreskin keratinocytes (HFK), E7 decreases the expression of miR-203, a miRNA involved in repression of proliferative capacity upon epithelial cell differentiation. The E7-mediated inhibition of miR-203 is followed by an overexpression of its primary target ΔNp63, an isoform of p63 highly expressed in undifferentiated basal keratinocytes and downregulated in normal non-proliferative cells. This process seems to occur through modulation of the mitogen-activated protein kinase (MAPK) signaling pathway, as a way to ensure cell proliferation and viral replication. Otherwise, miR-203 seems to be able to modulate HPV gene expression [50]. E7 also seems to upregulate the oncogenic miR-21 [51] and miR-27b [52]. Among miR-27b targets are the tumor suppressor *PLK2* [52] and PPARγ, a protein involved in the suppression of CC progression, thus leading to cell proliferation and invasion [53].

Besides E6 and E7, E5 oncoprotein also seems to modulate the expression of host miRNAs, although its role in regulating HPV replication and pathogenesis is poorly known. HPV-16 E5 induced transcriptional changes in HaCaT-E5 cells as compared to control cells, deregulating several miRNAs, such as miR-146a, miR-324-5p and miR-203, as well as their target genes [54]. HPV-16 E5 was also demonstrated to downregulate the tumor suppressor miR-196a, which increases HOXB8, a miR-196a target that is overexpressed in many cancers [55]. Thus, extensive data support the roles of E6 and E7 (and, to a less extent, of E5) interfering with the miRNA network.

Contrarily, miRNAs may also exert effects over E6 and E7, regulating HPV oncoproteins expression. One example is the effect of miR-375 over E6/E7. After transfection in HR-HPV-positive cells, miR-375 suppressed E6 and E7 proteins, controlled the CIP2A-MYC pathway (by inhibiting p21 repression) and decreased the hTERT through 14–3-3ζ regulation. MiR-375 also suppressed its direct target E6AP, a cellular protein that interacts with E6 for p53 degradation. As a result, p53, p21 and RB protein levels increased, in contrast to decreased cell proliferation [56]. Similar results were recently demonstrated by Wu et al. (2018), with overexpressed miR-375 inducing p53 and p21 expression, increasing caspase-3 and caspase-9 activities, and suppressing the expression of E6AP, IGF-1R, cyclin D1 and survivin proteins [57]. Other examples of miRNAs modulating HPV proteins are miR-125b (which is downregulated in cervical pre-cancerous lesions) suppressing HPV-16 DNA replication and synthesis [58], and miR-129-5p, an IFN-β induced miRNA, decreasing E6 and E7 expression [59]. Therefore, both miR-125b and miR-129-5p exert anti-HPV effects.

Together, these data provide new insights into the regulatory mechanisms between HPV and the miRNA network in host cells, evidencing the effects of HPV oncoproteins over the miRNA transcriptome, as well as of miRNAs over HPV global expression profile. The balance (or imbalance) among them is pivotal for HPV infection clearance or cervical carcinogenesis.

5. Extracellular vesicles and general features

EVs comprise a heterogeneous and complex set of structures released from all types of cells, delimited by a lipid bilayer, and transporting distinct molecules, such as nucleic acids, proteins, lipids and sugars, from their original cells [60,61]. Cellular communication is the

main function of these structures, in either an autocrine, paracrine or endocrine manner [62]. EVs are classified according to their mean size and mechanisms of origin, although there is still no consensus about the subdivision of EV main types [61, 63-65]. Exosomes correspond to vesicles with dimensions of 30–150 nm, while microvesicles (MV) and apoptotic bodies (AB) present dimensions of 50–1.000 nm and 50–5000 nm, respectively [61].

Exosomes are constantly secreted, usually from an endosomal origin [66]. In this process, early endosomes (EE) are formed from the invagination of part of the cell membrane. The population of EE that have not fused to pre-processed vesicles (and assigned their charge to degradation) turns into late endosomes, having part of their membrane invaginated. Known as multivesicular bodies (MVB), the late endosomes undergo maturation and formation of intraluminal vesicles. In this step, the participation of some protein complexes, such as the endosomal sorting complexes required for transport (ESCRTs) – consisting of four sub-complexes with distinct functions – is essential for selective vesicle loading of cellular molecules and for directing them to their destination [67,68]. Finally, some MVBs may fuse with the plasma membrane, releasing intraluminal vesicles into the extracellular space, now defined as exosomes [69]. Unlike exosomes, MV originate directly from the plasma membrane, through the formation of outward buds in specific sites of the membrane followed by fission and subsequent release of the vesicle into the extracellular space, with the plasma membrane suffering several molecular rearrangements at the sites of MV origin [70]. In turn, AB result from cellular apoptosis, initiating with the condensation of the nuclear chromatin, followed by membrane blebbing and disintegration of the cellular content into distinct membrane enclosed vesicles [71] (Fig. 2).

Different subtypes of EVs can be, to some extent, isolated from biological fluids, through distinct techniques, such as ultracentrifugation, immunoprecipitation and others [72], and differentiated from other protein aggregates. This is possible due to the presence of specific proteins on their surface, such as CD9, CD63 (essential for the formation of exosomes), Alix (an important regulator of the endolysosomal trafficking), TSG101 (one of the proteins that composes the ESCRT I sub-complex), among others [69,73]. However, since there are no available techniques to isolate highly pure populations of EVs, the International Society for Extracellular Vesicles (ISEV) encourages the use of the term “extracellular vesicle” as the generic term for particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate, i.e., do not contain a functional nucleus [74].

EVs are enriched with several non-coding RNAs, especially miRNAs. Four possible mechanisms involving miRNA sorting within EVs have been described. The first one was the nSMase2-dependent mechanism [75]. The second one is based on the correspondence between miRNA sequence motifs and modified heterogeneous ribonucleoproteins [76]. The third one highlights the importance of the modified 3′-end sequences of the transcripts for the encapsulation process [77], while the most recently proposed mechanism recognizes the importance of RISC complex proteins, such as Ago2, for the sorting of miRNAs into vesicles [78]. Furthermore, recent evidence indicates that Alix, an accessory protein of ESCRT, can be associated with Ago2 in order to favor the packaging of certain miRNAs into EVs [79] (Fig. 2).

Regarding EVs' biological effects, a great variety of physiological and pathological mechanisms has been demonstrated in the past few years. Due to EV's interactions with target cells (and their potential use as drug and gene delivery vehicles), these once discredited cell debris have recently emerged as mediators of intercellular communication (potentially delivering multiplexed information to surrounding tissues and systemically through the body) and promoters of disease pathogenesis. As cancer progression mediators, EVs may actively participate in cellular processes, such as proliferation, immune regulation, stromal remodeling, angiogenesis and metastasis [80-85].

EVs secreted by tumor cells are involved in evasion of the innate immune system since they have the ability to release miRNAs that can bind to Toll-like receptors and block the onset of adaptive immune responses [86]. Tumor-associated EVs may also act as immunosuppressive agents, mainly by modulating inflammatory reactions, by inducing the expression of inhibitory cytokines such as Interleukin-10, in addition to reducing the potential of T cell activation and natural killer cells [87–89]. Moreover, tumor-associated exosomes are involved in angiogenesis, and can be captured by endothelial cells, contributing to increased expression of factors such as vascular growth factor [87]. These tumor-associated EVs may be related to the establishment of metastatic niches in distant organs, since they have the capacity to attract tumor cells into the pre-metastatic niches [90,91], and can carry molecules that may contribute to genomic instability and/or that may epigenetically alter the chromatin conformational state in the acceptor cells [92]. Furthermore, other studies have also demonstrated the importance of EVs in the dormancy state prior to cancer recurrence [93].

On the other hand, EVs may help the immune system to fight cancer. Previous studies have demonstrated the importance of non-cancerous cell-released vesicles enriched in growth inhibitory miRNAs, which in turn may contribute to tumor clearance, avoiding tumor transformation [94]. Exosomes may present surface tumor antigens that can bind to specific ligands of dendritic cell surface, in order to initiate a complex and specific antitumor response [95].

For some gynecological cancers, tumor-secreted EVs also present different roles. Furthermore, the miRNA content of these vesicles reflects in clinical parameters and disease outcomes. In CC, as carcinogenesis is subsequent to infectious and inflammatory processes, EVs may be crucial for viral transmission and spreading, also being an essential part of tumor microenvironment establishment and disease progression [96,97]. In addition, the load of vesicular miRNAs (as well as of released cell-free miRNAs) seems to be profoundly influenced by HPV and its oncogenic proteins, as seen in sections 3 and 8 [33,98].

6. Overview of cellular miRNAs involved in SIL and CC

As presented before, evidences have shown the potential role of altered miRNA expression profiles in tumorigenesis. During the last decade, the involvement of miRNAs in cancer development became clearer, as well as their prognostic potential in several cancers, given their distinct signatures in different tumor types [7,99]. Moreover, distinct miRNA profiles seem to be found in different stages of precancerous lesions, presenting a diagnostic/prognostic value in the process of cervical transformation. Although further investigations are required in order to evaluate the application of miRNA profiles as contributory diagnostic/prognostic markers for SIL stages and CC, certainly a distinct and consistent signature is beginning to be set.

The pioneer work investigating cellular miRNAs and their association with CC was developed by Lui et al. (2007). Using a direct sequencing method, they have characterized the profiles of miRNAs of six human cervical carcinoma cell lines and five normal cervical samples. Out of 166 miRNAs expressed in normal cervix and cancer cell lines, 6 were differentially expressed between the two groups. Additionally, a reduced expression of miR-143 and an increased expression of miR-21 were reproducibly displayed in cancer samples, suggesting their potential value as tumor biomarkers in cervical carcinogenesis [100].

Shortly thereafter, a significant upregulation of the miRNA processor Drosha transcript (not observed for other genes of the miRNA processing pathway, *DGCR8*, *XPO5* and *DICER*), accompanied by a gain of chromosome 5p in over 50% of advanced cervical squamous cell carcinomas (SCC), was demonstrated. Importantly, Drosha overexpression appeared to be of functional significance, by modifying miRNA expression. Increased Drosha transcript levels in CC cells were associated with an increased expression of sixteen miRNAs, including miR-21, miR-29a, miR-31 and miR-203. SIL cases were also included in this study, but no significant miRNA expression deregulation, neither Drosha copy-number gain were observed in any SIL cases [101].

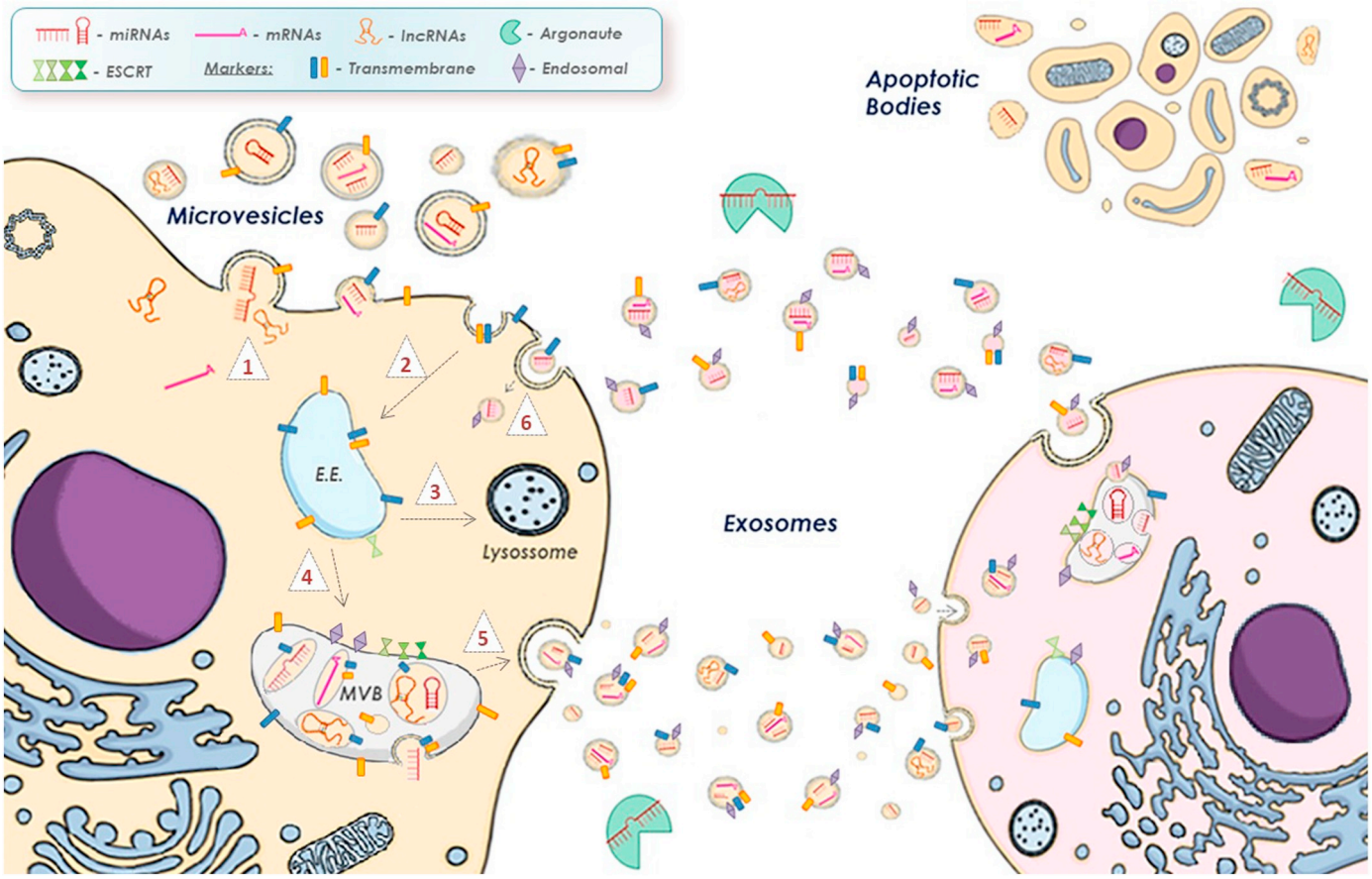

Fig. 2. Schematic representation of the biogenesis, cargo and mode of action of EVs. As nanoparticles released by cells, EVs carry distinct molecules, such as miRNAs, mRNAs, lncRNAs and proteins, thus, transporting molecular information to other cells. The EVs can be categorized by their origin and size as: AB, when originated by apoptotic cells (biogenesis not shown here); MV, released by folds of the cell membrane towards the extracellular space (1); or exosomes, released through the endosomal route, generally carrying specific endosomal molecules. The endosomal route is represented in the left cell according to the following steps: the process starts with cell membrane invagination and formation of the EE (2); EE can directly fuse with pre-processed vesicles and assign its charge to degradation (3) or it can turn into a late endosome (now called MVB) (4). In the MVB maturation process, its membrane folds inwards in order to form intraluminal bodies, and certain protein complexes (like ESCRT) select RNAs and proteins to be exported; finally, the MVB merges with the plasma membrane, releasing intraluminal vesicles (now called exosomes) into the extracellular space (5), where those exosomes can be incorporated by the same or other (neighboring or distant) cells, through circulation (6). AB, Apoptotic Bodies; EV, Extracellular Vesicles; MV, Microvesicles; MVB, Multivesicular Body; EE, Early Endosome; ESCRT, Endosomal sorting complexes required for transport. (Image source: Mind the Graph platform; https://mindthegraph.com).
Better as a 2-column fitting image.

Further on, an aberrant expression of distinct oncogenic and tumor-suppressive miRNAs in CC, which are important for cancer cell growth, was demonstrated. Investigating the expression profiles of miRNAs in HPV-16-positive CC cells, 174 deregulated miRNAs were found, including miR-21, miR-24, miR-27a, miR-205, and miR-193c. Significantly deregulated miRNAs were also observed in CC tissues, such as miR-143, and miR-145 (which were downregulated and showed to be tumor-suppressive), and miR-15b, miR-16, and miR-146a (which were upregulated, with the latter being involved in cell proliferation promotion). Therefore, they have paved the way to the idea that oncomiRs and tumor suppressor miRNAs may play an important role in cervical carcinogenesis [102].

Later, the miRNA expression profiles of a heterogeneous set of cervical tissues, including LSIL, HSIL and CC samples were evaluated. MiR-26a, miR-29a, miR-99a, miR-143, miR-145, miR-199a, miR-203, and miR-513 were found significantly downregulated in pre-neoplasic and malignant tissues in comparison with normal samples, while miR-148a, miR-302b, miR-10a, miR-196a and miR-132 were upregulated. Additionally, some miRNAs were upregulated in pre-neoplasic samples compared to normal cervix (e.g. miR-106a, miR-197, miR-16, miR-27a and miR-142-5p), but presented a relatively lower expression in CC, suggesting that they may play an important role in cervical cell abnormal transformation by HPV infection, being apparently not directly involved in the progression to a malignant state [103].

An altered miRNA expression in SIL and CC tissues, associated with chromosomal changes, was also observed. Significant differential expression during the consecutive stages of CC development was observed for 106 miRNAs, and an altered expression of five significantly differentially expressed miRNAs (miR-9, miR-15b, miR-28-5p, miR100 and miR-125b) was directly linked to frequent chromosomal alterations [104]. In addition, a transcriptome analysis of miRNAs and mRNAs for 24 cervical samples in different stages along SIL progression was performed, proposing an algorithm to detect miRNA-mRNA pairs with significant regulation change, constructing differential miRNA regulatory networks. Interestingly, the study provided clues for understanding SIL progression, presenting distinct differential miRNA regulatory networks from normal stage to LSIL, from LSIL to HSIL, and from HSIL to CC [105]. Likewise, several other relevant studies investigating deregulated miRNAs in SIL and CC samples were published, many confirming the involvement of previously described miRNAs. Most of these works were included in previously published reviews [9,106,107].

Taking into account all these reviews and a few other studies previously or subsequently published, consistent data on some deregulated

miRNAs in CC have arisen, as for miR-10a, miR-20a, miR-20b, miR-21, miR-31, miR-106a, miR-106b, miR-146a, miR-155, miR-196a, miR-200a, and miR-200b, which are overexpressed in CC tissues, and for miR-10b, miR-99a, miR-100, miR-125b, miR-143, miR-145, miR-195, miR-218, miR-376a, and miR-376b, which are downregulated in CC in comparison to normal cervix. As for SIL, although only few reports are available, some miRNAs have been described in more than one study with the same *status* of expression. MiR-10a, miR-21, miR-146a, and miR-196a were overexpressed in LSIL and HSIL tissues, while miR-99a, miR-143, miR-145, and miR-218 are downregulated in SIL samples. As mentioned before, these miRNAs have a similar pattern of expression as observed for CC, suggesting their early role in cervical carcinogenesis. Others, such as miR-27a, seem to be upregulated only in the pre-neoplasic tissue (compared to normal cervix), with reduced expression in CC stages (almost similar to normal cervix), thus, being mostly involved in cellular transformation [103]. In addition, other miRNAs like miR-155 are upregulated in HSIL and CC, but not in LSIL, suggesting their involvement during the latter stages of cervical carcinogenesis [108].

Otherwise, for some miRNAs, different studies have presented conflicting results in CC, as for miR-29a, miR-29c, miR-34a, miR-199a, miR-199b, miR-203, miR-378 and let-7c. For example, for miR-199a and miR-203, distinct patterns of expression were found. While the expression of miR-199a was found to be decreased in CC lines [102–104,109], it was overexpressed in other studies [110,111]. For miR-203, Muralidhar et al. (2007) and Witten et al. (2010) have demonstrated an overexpression of this miRNA in CC samples [101,112], while others showed a decrease in its expression [103,104,110,111,113–116]. For SIL, such contradictions are way less remarkable (due to the lack of studies), but the same contradictory pattern is observed, for example, for miR-378. While Wang et al. (2014) found miR-378 upregulated in LSIL, HSIL and CC [117], Wilting et al. (2013) demonstrated its downregulation in HSIL and CC [104]. Anyhow, these opposing findings may be due to several factors, such as differences in high-throughput platforms and methods used across laboratories, as well as the subtype of CC, the type of HPV involved, or if a single or a multiple infection is present [116,118].

Taken together, it is a fact that SIL and CC tissues have an altered miRNA expression pattern relatively to adjacent normal tissues. For SIL stages, such signature is still beginning to be revealed, especially because only a few studies included precancerous cervical specimens. For CC, however, the accumulating data concerning altered miRNA expression profiles are helping to form a solid miRNA signature. Despite these advances, it is important to highlight that such reliable and specific signatures are still under construction.

7. Cell-free miRNAs involved in SIL and CC

MiRNAs are found within cellular compartments, but also extracellularly, as circulating miRNAs. Initially, the idea of free miRNAs in distinct body fluids, as well as any RNA class, was unlikely accepted, especially considering the fact that such fluids are rich in ribonucleases. However, three pioneering studies have demonstrated the remarkable stability of endogenous extracellular miRNAs along with significant differences among cancer and control patients [119–121]. Despite these evidences, the precise mechanisms underlying circulating miRNA stabilization could not be defined at that point. To date, it is known that the stability of circulating miRNAs relies mostly on their carriers. For cell-free miRNAs, association with Ago protein, a part of the RISC, is essential for protecting the miRNA [122].

Accumulating data have shown that cell-free miRNAs are present in several body fluids, occurring both in physiological, as well as in pathological states [122]. Blood cells, such as leukocytes, erythrocytes and platelets, have been shown to secret several cell-free miRNAs. Platelets, in particular, seem to be an abundant source of cell-free miRNAs (i.e., miR-24, miR-191, miR-197, and miR-223). Besides that, in pathological conditions like cancer, tumor cells and other tissue- and organ-specific cells may represent relevant sources of released miRNAs (i.e., tissue-specific miRNAs associated with a particular disease). Therefore, a global miRNA profile expression in blood, for example, may represent a heterogeneous population of cell-free miRNAs from different origins, being a combination of blood, tumor and other organ/tissue-specific cells [123,124]. Likewise, this applies to CC and cervical pre-cancerous lesions, where cellular transformation had begun to occur [125]. Thus, defining a cell-free miRNA panel involved in SIL and CC has become an important issue, especially due to its minimally invasive nature.

During the last decade, researchers started to investigate cell-free miRNAs in the context of different stages of cervical carcinogenesis, initially focusing on CC stages. First, based on the fact that miR-218 was proved to be a tumor suppressor miRNA in several cancers, its *status* in CC samples was investigated. MiR-218 was detected in the serum of CC patients and normal age matched women, being significantly reduced in the serum of patients. Such reduction was significantly associated with clinicopathological characteristics, and lymph node metastasis (LNM) was associated with lower levels of miR-218, demonstrating its potential as a circulating prognostic marker for CC (and as a tumor suppressor miRNA), regulating tumor invasion and metastasis [126].

The potential of circulating miRNAs to predict LNM in patients with early-stage SCC was investigated by other groups [115,127,128] and, despite their focus on predicting LNM, they also brought information about potentially circulating miRNAs present in CC patients. Assuming that some miRNAs found in tissue may be used as fingerprints to predict potentially non-invasive biomarkers, promising candidates such as miR-1246, miR-20a, miR-2392, miR-3147, miR-3162-5p and miR-4484 appeared to be not susceptible to fluctuation in serum and tissue, presenting predictive values that were by far superior to that of SCC antigen (SCC-Ag), a current SCC marker [115]. MiR-20a and miR-203 also presented increased levels in CC patients compared to healthy donors, with miR-20a standing as a promising circulating biomarker for screening LNM [127]. MiR-205 was also significantly upregulated in CC patients, being correlated with poor tumor differentiation, LNM and increased tumor stage [128].

In addition, in order to predict CC at early stages, the expression profile of miRNAs in serum samples collected from CC patients and age- and ancestry-matched controls was investigated. After screening and validation phases, a panel composed by five miRNAs (miR-21, miR-25, miR-29a, miR-200a and miR-486-5p) was identified as a potential signature for CC detection at early stages. MiR-29a and miR-200a were shown to be upregulated in poorly differentiated cases compared with patients with well or moderately differentiated tumors, indicating tumor histological grade and progression stage [129].

Subsequently, knowing that miR-196a is upregulated in CC tissues and cervical cell lines, and given its ability to promote tumorigenesis in multiple types of cancers, the serum levels of miR-196a transcripts were evaluated in healthy volunteers and SIL patients. This was the first study involving SIL patients and revealed that miR-196a serum levels were higher in patients with CC and SIL compared to controls. In addition, miR-196a serum overexpression was associated with SIL grade and cervical transformation [130]. Likewise, aiming to establish cell-free miRNA serum signatures for diagnosing SIL and CC, other groups conducted multiple-phase studies (including initial, training and validation phases), revealing some promising candidates for early detection of cervical lesions, such as miR-2861 [131] and miR-1290 [132].

Aiming to establish a miRNA serum signature for early detection of SIL, the expression levels of certain miRNAs (miR-9, miR-10a, miR-20a and miR-196a) were measured in the serum samples of patients with SIL and healthy controls. The association between HPV infection *status*and miRNA levels was also evaluated. Results showed that the levels of all four miRNAs were significantly upregulated in the serum of SIL patients compared with healthy controls, constituting a useful panel of novel non-invasive biomarkers. High accuracy in discriminating SIL patients from healthy controls was observed, which might have great

clinical value for screening SIL [133].

Further on, knowing that miR-425-5p had been recently reported to be upregulated and to promote tumorigenesis in various cancer types, its prognostic significance in CC was evaluated. Including pairs of CC tissues and matched normal tissues, as well as serum samples from CC patients, benign cervical disease (cervical tissue with hyperplasia, showing some precancerous features) patients and healthy controls, miR-425-5p levels were assessed. Results demonstrated a significantly higher concentration of miR-425-5p in tumor tissues. Likewise, its serum levels were significantly higher in the CC group. Additionally, this expression was correlated with poor survival/poor diagnosis and was demonstrated as an independent prognostic biomarker for CC [134].

In addition, miR-486-5p*status* in serum samples and tissues (cancer tissues and adjacent normal tissues) obtained from CC patients and healthy donors was evaluated. MiR-486-5p was significantly over-expressed in CC patients' serum and tissues in comparison to control subjects (also a significant correlation between miR-486-5p expression in serum and in tissues was found), being qualified as a satisfactory diagnostic biomarker for CC. Moreover, using human CC cell lines (HeLa and SiHa), miR-486-5p was investigated as a potential regulator of CC development through the PI3K/Akt pathway by targeting PTEN (based on *in silico* studies). Basically, miR-486-5p stimulated cell proliferation, migration, and invasion through PTEN expression inhibition, as well as activation of the oncogenic PI3K/Akt pathway in CC, pointing to miR-486-5p as a novel diagnostic biomarker, as well as a plausible treatment target [135].

More recently, assuming that cervical mucus is an ideal material for profiling cervical neoplasia, the expression profiles of miRNAs in CC (and in precursor lesions) was analyzed in cervical mucus samples. Cervical mucus was collected from patients with normal cervix and CC cases (and with SIL). MiRNA array was performed and, among 2588 candidate miRNAs initially tested, four miRNAs (miR-126-3p, miR-20b-5p, miR-451a, and miR-144-3p) exhibited high fold changes in expression, being significantly upregulated in CC samples compared with normal cases. In particular, the fold change of miR-451a and miR-144-3p was significantly increased in CC cases (their expression levels also correlated with disease severity), suggesting that these miRNAs could be effective biomarkers for identifying CC (as well as high-grade precursor lesions) [136].

Likewise, other groups investigated deregulated cell-free miRNAs in SIL and/or CC. Table 1 and Table A.1 bring data about cell-free miRNAs involved in SIL and/or CC. In Table 1, we present only miRNAs that were shown deregulated by at least two articles in SIL and/or CC (miRNAs that were shown deregulated by only one paper are listed in Table A.1).

Differently from cellular miRNAs, the knowledge on cell-free miRNAs involved in cervical carcinogenesis is very limited, especially for SIL stages. If for cellular miRNAs this is a relevant issue, for cell-free miRNAs it is even more critical. However, although few, all these studies are helping to build a cell-free miRNA signature in SIL and CC, and may help the understanding of the role of miRNAs in cervical carcinogenesis from a global perspective.

8. EV-derived miRNAs involved in SIL and CC

In the past few years, EVs have emerged as new players in intercellular communication, acting as shuttles between cells. Recent advances highlight EVs' involvement in several pathophysiological processes as in cancer biology, and a growing body of evidence indicates that EVs can tightly modulate tumor progression [142]. They take part in cell-to-cell communication between cancer cells and neighboring cells, including fibroblasts and endothelial and immune cells. Since EVs are present in essentially all body fluids, containing bioactive molecules (such as mRNAs, miRNAs, DNA and proteins), they may reflect the pathological state of the cells of origin, standing as an enriched source of biomarkers. Moreover, the findings that these nanovesicles play critical roles in almost all aspects of cancer provide opportunities for their development as ideal diagnostic biomarkers, as well as therapeutic targets. Some studies revealed that the level of exosomes, for example, is elevated in the plasma of some patients with cancer as compared to healthy controls. Moreover, distinct sets of exosomal miRNAs seem to be present in different types of human cancers, also being positively correlated with cancer stage [143].

Tumor-derived EVs are garnering increasing attention because of their ability to act as small messengers that interact with target cells, directly via cell surface molecules, or after endocytosis and internalization of bioactive molecules. EVs may exert direct effects through horizontal (lateral) transfer of bioactive molecules into recipient cells, or indirect effects by remodeling the cancer microenvironment. Interestingly, the transfer of EV's content from a given cell to a recipient cell appears to readily affect and reprogram the phenotype of these cells [144].

In recent years, an exponential number of studies involving EV-derived miRNAs has emerged. For cancer screening and diagnosis, EV-derived miRNAs from liquid biopsy may be advantageously used instead of cellular miRNAs, for example, especially considering their potential as minimally invasive and early detection biomarkers in different cancer types, as well as their promising ability to give an overview of the heterogeneity of both the tumor and the microenvironment.

Differently from cellular or cell-free miRNAs, the research involving EV-derived miRNAs has recently started. Indeed, to the best of our knowledge, until the beginning of this year, only one research group has investigated the levels of miRNAs in CC-derived exosomes. Liu et al. (2014) determined the association of two miRNAs (previously demonstrated to be involved in cervical oncogenesis) with CC-derived exosomes. Exosomes were isolated from the cervicovaginal lavages specimens from patients with CC, HPV-positive subjects and HPV-negative subjects. The abundance of exosomes and exosomal miRNA levels were addressed. The levels of exosomal miR-21 and miR-146a were significantly higher in the cervicovaginal lavage specimens of CC subjects in comparison with HPV-positive and HPV-negative subjects. In addition, differential exosomal miR-21 levels were also found between HPV-positive and HPV-negative groups, thus, suggesting a significant contribution of HPV infection for the up-regulation of miR-21 in cervical tissue. Moreover, an increase in the abundance of exosomes was also observed in the cervicovaginal lavage specimens of women with CC [145], as shown in Table 2.

Besides that, a few *in vitro* studies, mostly investigating EV-derived miRNA profile changes in cancer cell lineages expressing (or not) E6 and E7 oncoproteins have been published (Table 2). The first one did not involve miRNAs, but was a pioneer study. The effects of silencing the viral *E6/E7* genes on survivin (which served as a proper protein model) and over the amounts of exosomes released from HeLa cells (containing HPV-18) were evaluated. By investigating an exosomal-enriched fraction, they observed that silencing endogenous *E6/E7* expression strongly reduced both cellular and microvesicular concentrations of survivin, suggesting an intracellular HPV-induced change in exosomes secreted from HPV-positive cancer cells. Moreover, an increase in the overall amounts of exosomes released from HPV-positive cancer cells was observed, which was linked to an induction of cellular senescence and stimulation of p53 target genes. Therefore, HPV *E6/E7* expression seems to influence both the amounts and the contents of exosomes secreted from HeLa cells [146].

Later, by deep sequencing analyses, the same group investigated if *E6/E7* expression would influence the cellular miRNA network. After silencing endogenous *E6/E7* expression in HeLa cells, a deregulation in ten of the 52 most abundant intracellular miRNAs was observed (with a downregulating of miR-17-5p, miR-186-5p, miR-378a-3p, miR-378f, miR-629-5p and miR-7-5p, and an upregulating of miR-143-3p, miR-23a-3p, miR-23b-3p and miR-27b-3p), along with a similar pattern for SiHa cells (containing HPV-16). Additionally, they identified an E6/E7-

Table 1
Cell-free miRNAs involved in different stages of cervical carcinogenesis (SIL/CC).

miRNA	LSIL	HSIL	CC
	Status/sample/population (Reference)	*Status*/sample/population (Reference)	*Status*/sample/population (Reference)
hsa-let-7a-5p (hsa-let-7a)	–	–	Down/Serum/C [115][a]
	–	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]
miR-9-5p (hsa-miR-9)	Up/Serum/C [133][a]	Up/Serum/C [133][a]	Up/Serum/C [131][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-10a-5p (hsa-miR-10a)	Up/Serum/C [133][a]	Up/Serum/C [133][a]	–
	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]
hsa-mir-17-5p (hsa-miR-17)	–	–	Down/Serum/C [131][a]
	–	–	Up/Mucus/J [136][b]
hsa-mir-20a-5p (hsa-miR-20; −20a)	Up/Serum/C [133][a]	Up/Serum/C [133][a]	Up/Serum/C [115][b]
	–	–	Up/Serum/C [127][a]
hsa-mir-21-5p (hsa-miR-21)	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]	Up/Mucus/J [136][b]
	–	–	Up/Serum/C [137][a]
	–	–	Up/Serum/C [129][b]
hsa-mir-29a-3p (hsa-miR-29a)	–	–	Up/Serum/C [129][b]
	–	–	Up/Serum/C [131][a]
hsa-mir-92a-3p (hsa-miR-92; −92a)	Up/Serum/C [138][a]	Up/Serum/C [138][a]	Up/Serum/C [138][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-101-3p (hsa-miR-101)	–	–	Up/Serum/C [139][a]
	–	–	Up/Serum/C [129][a]
hsa-mir-103a-3p (hsa-miR-103; −103a)	–	–	Down/Serum/C [137][a]
	–	–	Down/Serum/C [131][a]
hsa-mir-106a-5p (hsa-miR-106a)	–	–	Down/Serum/C [131][a]
	–	–	Up/Mucus/J [136][b]
hsa-mir-122-5p (hsa-miR-122a; −122)	–	–	Up/Serum/C [131][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-132-3p (hsa-miR-132)	–	–	Up/Serum/C [131][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-139-3p (hsa-miR-139-3p)	–	–	Down/Serum/C [131][a]
	–	–	Down/Serum/C [137][a]
hsa-mir-141-3p (hsa-miR-141)	–	–	Up/Serum/C [137][a]
	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]	Up/Mucus/J [136][b]
hsa-mir-144-3p (hsa-miR-144)	–	–	Down/Serum/C [131][a]
	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]	Up/Mucus/J [136][b]
hsa-mir-155-5p (hsa-miR-155)	–	–	Up/Serum/C [131][a]
	Up/Mucus/J [136][b]	Up/Mucus/J [136][b]	Up/Mucus/J [136][b]
hsa-mir-191-5p (hsa-miR-191)	–	–	Up/Serum/C [129][a]
	–	–	Down/Serum/C [131][a]
hsa-mir-195-5p (hsa-miR-195)	–	–	Down/Serum/C [131][c]
	–	–	Down/Serum/C [137][a]
hsa-mir-196a-5p (hsa-miR-196a)	Up/Serum/C [130][a]	Up/Serum/C [130][a]	Up/Serum/C [130][a]
	Up/Serum/C [133][a]	Up/Serum/C [133][a]	–
hsa-mir-200c-3p (hsa-miR-200c-3p)	–	–	Up/Serum/C [131][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-203a-3p (hsa-miR-203a; −203)	–	–	Up/Serum/C [127][a]
	–	–	Up/Serum/C [131][a]
hsa-mir-205-5p (hsa-miR-205)	Up/Mucus/J [136][a]	Up/Mucus/J [136][a]	Up/Mucus/J [136][b]
	–	–	Up/Serum/C [137][a]
	–	–	Up/Plasma/C [140][a]
	–	–	Up/Serum/C [128][a]
hsa-mir-212-3p (hsa-miR-212)	–	–	Up/Serum/C [137][a]
	–	–	Down/Serum/C [131][a]
hsa-mir-214–3p (hsa-miR-214)	–	–	Up/Serum/C [131][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-218-5p (hsa-miR-218)	–	–	Down/Serum/C [126][a]
	–	–	Down/Serum/C [141][a]
hsa-mir-370	–	–	Up/Serum/C [131][a]
	–	–	Up/Serum/C [137][a]
hsa-mir-425-5p (hsa-miR-425)	–	–	Down/Serum/C [131][a]
	–	–	Up/Serum/C [134][a]
hsa-mir-451a (hsa-mir-451)	Up/Mucus/J [136][a]	Up/Mucus/J [136][b]	Up/Mucus/J [136][b]
	–	–	Down/Serum/C [115][a]
hsa-mir-486-5p (hsa-miR-486)	–	–	Up/Serum/C [135][a]
	–	–	Up/Serum/C [129][b]
	–	–	Up/Serum/C [137][a]
hsa-mir-1246	–	–	Up/Serum/C [115][b]
	–	–	Up/Serum/J [132][c]

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CC, cervical cancer; Up, upregulated; Down, downregulated; C, Chinese; J, Japanese. [a]Results obtained directly by microarray, or next-generation sequencing, or RT-qPCR; [b]Results obtained by microarray, or next-generation sequencing, or RT-qPCR and validated by RT-qPCR; [c]Results obtained by microarray, or next-generation sequencing, or RT-qPCR and validated by at least two sets of RT-qPCR.
MiRNA nomenclature was based on miRBase 22.1 IDs. Note that almost all herein referred miRNAs present the -3p or -5p identification, while the authors do not reference the strand of origin in the article. Such nomenclature was based on miRBase 22.1 IDs; for example, miR-122 was simply referred to as miR-122 in the respective article, while in miRBase miR-122 and miR-122a are previous IDs for hsa-miR-122-5p. For some miRNAs, such as miR-370, miR-451a, and miR-1246, there is still no standardization concerning the strand of origin. All the above-mentioned miRNAs are hsa-miRNAs (*Homo sapiens* miRNAs).

dependent seven-miRNA signature in exosomes secreted from HeLa cells (with a downregulation of let-7d-5p, miR-20a-5p, miR-378a-3p, miR-423-3p, miR-7-5p and miR-92a-3p, and an upregulation of miR-21-5p), which seems to be linked to the control of cell proliferation, senescence and apoptosis [98].

Additionally, the role of HPV oncoproteins in keratinocytes transduced with E6/E7 from mucosal HPV-16 or cutaneous HPV-38 (K16 and K38) was investigated. In order to study miRNA involvement in HPV-associated tumorigenesis and analyze the differences between keratinocytes transformed by mucosal HPV-16 and by cutaneous HPV-38, a miRNA array analysis of 384 human miRNAs was performed, and a culture of HFK was used as a control. Several miRNAs were found to be deregulated in K16 and K38 cells. Besides that, HPV-16 and/or HPV-38 E6 and E7 single proteins seem to be able to modify the expression of a few miRNAs involved in the tumorigenesis, in particular of miR-18a, miR-19a, miR-34a, and miR-590-5p. Interestingly, E6 and E7 mRNAs were found in exosomes isolated from HPV-positive cells, supporting a transfer of HPV oncoproteins by exosomes [147].

More recently, it was also demonstrated that the expression of HPV oncoproteins seems to alter the expression of different secreted miRNAs. Basically, the researchers investigated how HPV-16 *E6/E7* oncogene expression in primary human epithelial cells alters miRNA expression in cells and derived EVs. The expression of a panel of 68 cancer-related miRNAs was evaluated in cells, in exosome-enriched EVs released by HPV-16 *E6/E7*-expressing HFKs, and in matched-control, vector-transduced HFK. Most of the analyzed miRNAs presented a similar pattern of expression both in cells and in exosome-enriched EVs, and were regulated by E6/E7. Some, however, were differently found among them, suggesting a selective packaging in exosome-enriched EVs secreted by HPV-16 *E6/E7*-expressing cells. Indeed, seven miRNAs showed a different abundance in exosome-enriched EVs. Among these selectively packaged miRNAs, five were found to be involved in apoptosis and necrosis inhibition (miR-16-5p, miR-200b-3p, miR-222-3p, miR-320a, and miR-378a-3p) [148].

Together, these data demonstrate that HPV *E6/E7* oncogenes expression can profoundly affect the expression of many miRNAs both intracellularly (as in section 4), as well as packaged in EVs. In addition, the expression patterns of cellular and EV-derived miRNAs seem to vary, suggesting a distinct and important function for released miRNAs. Such alterations of EV-derived miRNA levels may be involved in cancer development and maintenance, highlighting the importance of understanding the crosstalk between HPV and the miRNA network [149]. Moreover, besides understanding such crosstalk, further research investigating EV-derived miRNAs in SIL and CC are of immediate need.

9. Perspectives: potential biomarkers in cervical carcinogenesis

In spite of their “tiny” size, miRNAs are now undoubtedly known as important players in cancer establishment, development and progression. Their importance in cervical microenvironment, either following HPV's infection, or in SIL development and/or progression to CC, is undeniable. Accumulating evidences demonstrating miRNA's involvement in SIL and/or CC consolidate such fact.

As discussed before, HPV itself originates some viral miRNAs, as well as exerts effects on the host's miRNA network, deregulating human miRNAs in a direct or indirect E6/E7-dependent way. Thus, after HPV infection, an active interaction between the virus and the human genome is expected. Indeed, some human miRNAs seem to be strongly associated with HPV infection, such as cellular miR-9 [46], cellular miR-21 [41,51,148] and EV-derived miR-21 [145,148], cellular miR-34a [39,147,148] and EV-derived miR-34a [148], cellular miR-146a [54] and EV-derived miR-146a [145]. Despite these evidences, information on these and other miRNAs is very limited, hindering the definition of a panel of miRNAs able to characterize HPV infection *per se*.

Part of the explanation is based on the fact that such expression does not seem to be an isolated event, following viral infection. Therefore, it may be separated from events that come subsequently, as dysplastic

Table 2
Summary of studies investigating EV-derived miRNAs involved in cervical carcinogenesis or HPV-induced tumorigenesis (*in vivo* or *in vitro*).

Ref.	Tissue/cell line	Basic strategy	Main findings
[145]	Cervical vaginal lavage	Investigation of exosome-derived miRNA levels (miR-21 and miR-146a) in patients with CC, HPV-positive and HPV-negative subjects (RT-qPCR)	MiR-21 (miR-21-5p) and miR-146a (miR-146a-5p) were upregulated in CC in comparison with the other groups, and in HPV-positive group in comparison with HPV-negative group.
[146]	HeLa	Investigation of the influence of silenced *E6/E7* genes on released exosome concentration and cellular/microvesicular content (Immunoblotting)	Silencing *E6/E7* resulted in a decrease in cellular/microvesicular concentration of survivin and increase in exosome-enriched fraction amounts (as a result of cellular senescence/p53 target genes activation).
[98]	HeLa/SiHa	Investigation of the influence of silenced *E6/E7* genes on miRNA network (Deep Sequencing)	MiR-17-5p, miR-186-5p, miR-378a-3p, miR-378f, miR-629-5p and miR-7-5p were downregulated in *E6/E7*-silenced HeLa cells. MiR-143-3p, miR-23a-3p, miR-23b-3p, and miR-27b-3p were upregulated in *E6/E7*-silenced HeLa cells. Similar patterns were observed for SiHa cells.
[147]	HFK	Investigation of the effects of HPV oncoproteins on the miRNA network of keratinocytes transduced with *E6/E7* from mucosal HPV-16 or cutaneous HPV-38 (K16 and K38) (TaqMan microRNA array)	MiR-10a (miR-10a-5p), miR-18a (miR-18a-5p), miR-19a (miR-19a-3p), miR-19b (miR-19b-3p), miR-21 (miR-21-5p), miR-29b (miR-29b-3p), miR-30b (miR-30b-5p), miR-98 (miR-98-5p), miR-99a (miR-99a-5p), miR-182 (miR-182-5p), miR-194 (miR-194-5p), and miR-590-5p were upregulated in both cell lineages transduced with *E6/E7* in comparison with non-transduced HFK. MiR-34a (miR-34a-5p) was upregulated in K38 cells, and downregulated in K16 cells, in comparison with non-transduced HFK. MiR-222 (miR-222-3p) was upregulated in K16 cells, while miR-375 was downregulated in K38 cells, in comparison with non-transduced HFK.
[148]	HFK	Investigation of the effects of HPV oncoproteins on miRNA levels in EVs of keratinocytes transduced with HPV-16 *E6/E7* (FirePlex® miR Oncology Assay)	MiR-16-5p, miR-200b-3p, miR-205-5p, miR-222-3p, miR-320a, miR-378a-3p were differentially expressed in cellular/EV compartments. MiR-19a-3p, miR-21-5p, miR-25-3p, miR-34a-5p, miR-93-5p, miR-106b-5p, miR-107, miR-148b-3p, miR-155-5p, miR-182-5p, miR-195-5p, miR-218-5p, miR-335-5p, miR-375, miR-376c-3p, miR-625-3p, and miR-652 were similarly expressed in cellular/EV compartments.

Ref., Reference; RT-qPCR, Reverse transcription-polymerase chain reaction; HFK, Human foreskin keratinocytes. MiRNA nomenclature was based on miRBase 22.1 IDs. Note that almost all herein referred miRNAs present the -3p or -5p identification, while the authors do not reference the strand of origin in the article. Such nomenclature was based on miRBase 22.1 IDs; for example, miR-21-5p was simply referred to as miR-21 in the respective article. For some miRNAs, such as miR-375 there is still no standardization concerning the strand of origin. All the above-mentioned miRNAs are hsa-miRNAs (*Homo sapiens* miRNAs).

changes in the cervical epithelium, which may also interfere with the cellular and circulating miRNA profiles. Actually, an overlap seems to occur throughout the process of cancer induction. That is one of the reasons why some of these miRNAs, among many others, are described at cellular and extracellular levels, but associated with SIL or CC degrees, and not with HPV infection as an isolated event. In order to define reliable HPV infection-related miRNAs as biomarkers, more experiments involving patients with HR-HPV-positive *status*and free from cervical disease (SIL or CC), as well as their matched controls, are required.

On the other hand, defining promising miRNAs (or specific miRNA signatures) to characterize SIL and CC stages seems more tangible at the present moment (especially concerning cellular miRNAs), despite all the remaining difficulties involved. First, defining a reliable biomarker involves knowing its basic nature – a cellular, biochemical, or molecular (proteomic, genetic, and epigenetic) alteration that is indicative of normal biological or pathogenic processes, or pharmacological responses to a therapeutic intervention, and that are measurable in biological material such as human tissues or fluids. In practice, they are biological 'events' that can aid in understanding prediction, cause, diagnosis, progression, regression, or treatment outcome of a certain disease [150]. In cancer research and detection (and we can extend this to pre-cancerous stages), a biomarker refers to a substance or process that is indicative of the presence of cancer (lesion) in the body. It might be either a molecule secreted by malignancy (or as a consequence of dysplastic changes) itself or a specific response of the body to the presence of cancer (lesion) [151]. Second, it is important to understand that characteristics such as reliability, validity, sensitivity, and specificity need to be intrinsically associated to these biomarkers.

To date, we have hundreds of articles reporting information about deregulated expression of cellular miRNAs in SIL and/or CC, and only a few regarding either cell-free or EV-derived circulating miRNAs. Contrarily, although a wide-range of miRNAs has been described, most of these results are not reproducible, frequently lacking solid reliability, validity, sensitivity, and specificity. This, together with the use of different techniques and analysis methods, as well as different populations and samples employed, are impairing the settlement of some miRNAs as trustworthy biomarkers for SIL and/or CC.

However, looking carefully at published evidences, it is possible to recognize some patterns. For cellular miRNAs (see section 6), some candidate miRNAs have already been identified, such as miR-10a, miR-21, miR-146a, and miR-196a – upregulated in SIL and CC cases – and miR-99a, miR-143, miR-145, and miR-218 – downregulated in SIL and CC cases – thus serving as cervical disease biomarkers. Other miRNAs, such as miR-27a, may serve as early stage miRNA biomarkers, being upregulated only in SIL stages (and not in CC) [103], while others may serve as late stage miRNA biomarkers, among which stand out miR-155 (upregulated in HSIL and CC stages) [104,116], and miR-195 (downregulated in HSIL and CC) [104,118]. Actually, there is still a wide range of cellular miRNAs needing further investigation in order to have clarified their *status* in cervical carcinogenesis.

For circulating miRNAs, it is also possible to identify some promising miRNAs (or specific miRNA signatures), although fewer studies are available so far. Here, we only discuss and present as promising circulating biomarkers those miRNAs that were shown deregulated by at least two studies in SIL and/or CC samples. Assuming that we found some miRNAs that present similar patterns of expression at the cellular and extracellular (as cell-free and/or EV-derived miRNAs) levels, we therefore highlight them as promising circulating miRNAs. Examples are miR-9, miR-10a, miR-20a, miR-92a, miR-141, and miR-196a, all found upregulated as cellular [9,106,107] and cell-free [115,127,130,131,133,136,137] miRNAs in SIL stages and CC, suggesting their potential as non-invasive biomarkers for cervical disease. Indeed, as seen before, a miRNA panel composed of miR-9, miR-10a, miR-20a and miR-196a showed high accuracy in discriminating SIL patients from controls, indicating its potential clinical value for screening SIL [133].

Additionally, miR-132, miR-486, and miR-1246 were found upregulated as cellular [9,106,107] and cell-free [115,129,131,132,135,137] miRNAs, but only in CC, thus implying a potential role for these miRNAs as non-invasive biomarkers for CC. Interestingly, besides being qualified as a suitable diagnostic biomarker for CC (due to significant correlation between its cellular and extracellular levels), miR-486 was found to stimulate cell proliferation, migration, and invasion through PTEN expression inhibition and activation of the PI3K/Akt pathway in CC, also standing as a reasonable treatment target [135]. Additionally, miR-146a also stands as a promising non-invasive biomarker for CC, since it was found upregulated within CC tissues [21,104], as well as in EVs [145].

Likewise, cellular miR-21, cell-free miR-21 and EV-derived miR-21 were also found upregulated in CC (and upregulated in LSIL and HSIL tissues, as well as cell-free miRNAs) [9,106,107,129,136,137,145]. Despite its broad expression, as miR-21 stands as a key player in cancer (and disease, in general) [152], it may not properly meet the requirements of a specific biomarker for SIL and/or CC. Additionally, a few miRNAs were described only as cell-free miRNAs: miR-103a, miR-122, miR-139, miR-214, and miR-370, all found upregulated in CC stages [131,137]. In order to evaluate if they are present within cellular compartments and/or secreted within EVs, or if they are specifically cell-free miRNAs, more studies are required. Thus, here we identify promising circulating miRNAs (or a specific miRNA signature), which may stand as potential future biomarkers of cervical carcinogenesis. Fig. 3 summarizes schematically the main findings on circulating miRNAs in LSIL, HSIL and CC stages, found in both cervical and blood compartments. Some cellular miRNAs are also shown, although many were not represented here.

Furthermore, apart from defining potential biomarkers for cervical carcinogenesis (which is the main goal of this review), we aimed to establish connections and discrepancies between what is found within cellular and extracellular compartments – as cellular, cell-free and EV-derived miRNAs. Similar patterns were already described above. Opposing ones are mentioned bellow. For example, the expression patterns of let-7a, miR-17, miR-191, miR-203, miR-212, miR-425, and miR-451a are known to vary at the cellular level among different studies. Such variation is also observed when the cellular and cell-free levels of these miRNAs are compared. MiR-425 (miR-425-5p), for example, seems to be an important miRNA in tumorigeneses in different cancers, with some studies showing its potential as a biomarker. Sun et al. (2017) have demonstrated a significant correlation between miR-425 levels in tissue and serum from CC patients (upregulated in both), defining this miRNA as an independent prognostic biomarker for CC [134]. Nevertheless, as other studies are in disagreement with the above-mentioned one, new data are required to confirm or disregard miR-425as a biomarker.

Another interesting example is the expression of miR-205, which varies according to cellular and extracellular compartments and depending on cervical disease stage. Different studies have shown an upregulation of cellular miR-205 in CC [102,104,108,109,112,116], while another has demonstrated a relatively decreased expression of cellular miR-205 in the transition from normal cervix to atypical dysplasia and its increased expression in the transition from atypical dysplasia to CC [103]. Cell-free miR-205, in turn, was found upregulated in LSIL, SIL and CC in different populations and samples (mucus, plasma and serum) [128,136,137,140]. And curiously, EV-derived miR-205 was found downregulated in EVs released from keratinocytes transduced with HPV-16 E6/E7 [148]. For miR-218, also a contradictory but interesting pattern was observed.

Additionally, cellular miR-218 was extensively described as downregulated in LSIL, SIL and CC [21,44,104,118,141], and has been considered as a tumor suppressor miRNA. Accordingly, cell-free miR-218 was found downregulated in CC samples [126,141]. However, EV-derived miR-218 was found upregulated in EVs released from

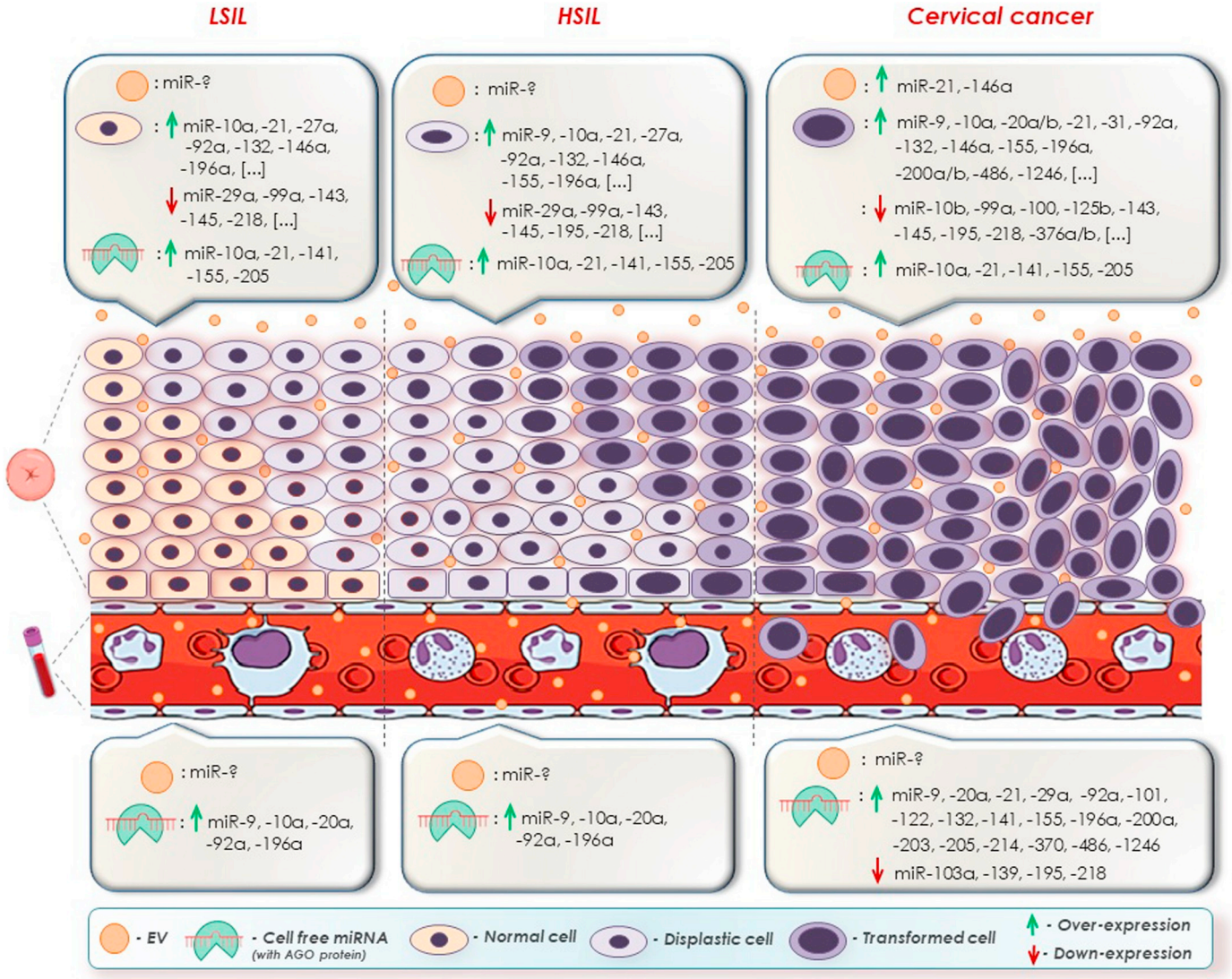

Fig. 3. MiRNAs as potential biomarkers for SIL and/or CC in cervical and blood compartments. Illustrative overview of promising miRNAs described in LSIL, HSIL and CC stages, as cellular (upper panels), cell-free or EV-derived miRNAs, within cervical and blood samples (lower panels). As cervical dysplastic changes and cellular transformation occur, from SIL to CC, different miRNAs are expressed. Here, despite our main focus on circulating miRNAs, we represent some of the most common cellular miRNAs described, in order to reinforce connections (as well as gaps) between what is found within cellular and extracellular compartments. Only miRNAs that with concordant patterns of expression are represented here (miRNAs presenting conflicting results were not included). SIL, squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CC, cervical cancer. (Image source: Mind the Graph platform; https://mindthegraph.com).

keratinocytes transduced with HPV-16 E6/E7 (and was similarly found in the cellular compartment, as well as within EVs) [148]. In order to confirm and understand these opposing findings, more studies are definitely needed. Despite that, miR-205 and miR-218 seem reproducible as cell-free miRNAs in CC [126,128,136,137,140,141].

For sure, concerning circulating miRNAs, only a scarce amount of information is available. Thus, contradictory patterns like those above-mentioned may be a result of insufficient data, or indeed have a biological purpose. Regardless, defining similar and opposing patterns is of urgent need. To date, it is still uncertain if a cell releases cell-free miRNAs in a programmed and selective way, or if this release occurs 'by chance' or as a result of cellular death. If accidentally, does it still mean something? Can these biomarkers be useful somehow? Concerning EV-derived miRNAs, there is a consensus that they are selectively packaged into EVs. Thus, adopting EVs as non-invasive biomarkers may represent a more appropriate approach in comparison to cell-free miRNAs. Is that really true? All these questions are still unanswered.

Finally, as challenges and limitations concerning circulating miRNA research, it is important to mention that almost all the studies investigating circulating miRNAs involves samples from the Chinese population, with a few involving Japanese participants. Thus, it is difficult to establish 'global' circulating biomarkers in cervical carcinogenesis. Other limitations are the use of different techniques and/or analysis methods, as well as the lack of studies involving other biological fluids.

10. Conclusions

MiRNAs' involvement in HPV infection, SIL development and CC progression is becoming more evident each day, as well as their potential use as non-invasive biomarkers. Taking that for granted, defining a circulating miRNA panel involved in SIL and/or CC has become an important issue in recent years, perhaps being even more important than defining a cellular miRNA signature, especially considering that obtaining cervical tissue involves an invasive procedure. Certainly, this is really a research field under recent construction - if this was true for cellular miRNAs, it is even more evident for circulating miRNAs.

Despite the numerous challenges, finding new molecular non-invasive biomarkers, with high sensitivity and specificity is strictly necessary. Hence, defining promising circulating miRNAs (or specific miRNA signatures) from biological fluids specimens may be useful for screening, diagnosis, prognosis, and clinical monitoring of drug responses, as well as for predicting SIL or CC risk. In addition, such approach may allow an early intervention of precancerous stages, such as LSIL and HSIL, yielding a higher chance for cure.

Funding sources

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior 001).

Acknowledgements

We acknowledge the Graduate Program of Genetics of the Department of Genetics from the Federal University of Paraná.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbcan.2019.08.001.

References

[1] WHO, Cervical cancer, WHO, https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/, (2018) , Accessed date: 14 January 2019.
[2] L. Denny, Cervical cancer: prevention and treatment, Discov. Med. 14 (2012) 125–131 http://www.ncbi.nlm.nih.gov/pubmed/22935209 , Accessed date: 14 January 2019.
[3] C.A. Moody, L.A. Laimins, Human papillomavirus oncoproteins: pathways to transformation, Nat. Rev. Cancer 10 (2010) 550–560, https://doi.org/10.1038/nrc2886.
[4] D. Subramanya, P.D. Grivas, HPV and cervical cancer: updates on an established relationship, Postgrad. Med. 120 (2008) 7–13, https://doi.org/10.3810/pgm.2008.11.1928.
[5] Y. Huang, X.J. Shen, Q. Zou, S.P. Wang, S.M. Tang, G.Z. Zhang, Biological functions of microRNAs: a review, J. Physiol. Biochem. 67 (2011) 129–139, https://doi.org/10.1007/s13105-010-0050-6.
[6] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell. 136 (2009) 215–233, https://doi.org/10.1016/j.cell.2009.01.002.
[7] J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz, T.R. Golub, MicroRNA expression profiles classify human cancers, Nature. 435 (2005) 834–838, https://doi.org/10.1038/nature03702.
[8] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866, https://doi.org/10.1038/nrc1997.
[9] Y. He, J. Lin, Y. Ding, G. Liu, Y. Luo, M. Huang, C. Xu, T.-K. Kim, A. Etheridge, M. Lin, D. Kong, K. Wang, A systematic study on dysregulated microRNAs in cervical cancer development, Int. J. Cancer 138 (2016) 1312–1327, https://doi.org/10.1002/ijc.29618.
[10] J. Ardila-Molano, M. Vizcaíno, M.L. Serrano, Circulating microRNAs as potential cancer biomarkers, Rev. Colomb. Cancerol. 19 (2015) 229–238, https://doi.org/10.1016/J.RCCAN.2015.08.002.
[11] J. Doorbar, N. Egawa, H. Griffin, C. Kranjec, I. Murakami, Human papillomavirus molecular biology and disease association, Rev. Med. Virol. 25 (Suppl. 1) (2015) 2–23, https://doi.org/10.1002/rmv.1822.
[12] D. Forman, C. de Martel, C.J. Lacey, I. Soerjomataram, J. Lortet-Tieulent, L. Bruni, J. Vignat, J. Ferlay, F. Bray, M. Plummer, S. Franceschi, Global burden of human papillomavirus and related diseases, Vaccine. 30 (2012) F12–F23, https://doi.org/10.1016/j.vaccine.2012.07.055.
[13] N. Muñoz, F.X. Bosch, S. de Sanjosé, R. Herrero, X. Castellsagué, K.V. Shah, P.J.F. Snijders, C.J.L.M. Meijer, International Agency for Research on Cancer Multicenter Cervical Cancer Study group, epidemiologic classification of human papillomavirus types associated with cervical cancer, N. Engl. J. Med. 348 (2003) 518–527, https://doi.org/10.1056/NEJMoa021641.
[14] C.B.J. Woodman, S.I. Collins, L.S. Young, The natural history of cervical HPV infection: unresolved issues, Nat. Rev. Cancer 7 (2007) 11–22, https://doi.org/10.1038/nrc2050.
[15] D.A. Galloway, L.A. Laimins, Human papillomaviruses: shared and distinct pathways for pathogenesis, Curr. Opin. Virol. 14 (2015) 87–92, https://doi.org/10.1016/j.coviro.2015.09.001.
[16] K.P. Braaten, M.R. Laufer, Human Papillomavirus (HPV), HPV-Related Disease, and the HPV Vaccine, Rev. Obstet. Gynecol. 1 (2008) 2–10 http://www.ncbi.nlm.nih.gov/pubmed/18701931 , Accessed date: 27 September 2018.
[17] D.D. Davey, M.H. Neal, D.C. Wilbur, T.J. Colgan, P.E. Styer, D.R. Mody, Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists Interlaboratory comparison program in cervicovaginal cytology, Arch. Pathol. Lab. Med. 128 (2004) 1224–1229, https://doi.org/10.1043/1543-2165(2004)128<1224:BIARRP>2.0.CO;2.
[18] B.S. Apgar, L. Zoschnick, T.C. Wright, The 2001 Bethesda system terminology, Am. Fam. Physician 68 (2003) 1992–1998 http://www.ncbi.nlm.nih.gov/pubmed/14655809 , Accessed date: 27 September 2018.
[19] M. Zsemlye, High-grade cervical dysplasia: pathophysiology, diagnosis, and treatment, Obstet. Gynecol. Clin. N. Am. 35 (2008) 615–621, https://doi.org/10.1016/j.ogc.2008.09.008.
[20] S.C. Tan, R. Ankathil, Genetic susceptibility to cervical cancer: role of common polymorphisms in apoptosis-related genes, Tumour Biol. 36 (2015) 6633–6644, https://doi.org/10.1007/s13277-015-3868-2.
[21] G. Sharma, P. Dua, S.M. Agarwal, A comprehensive review of dysregulated miRNAs involved in cervical cancer, Curr. Genomics. 15 (2014) 310–323, https://doi.org/10.2174/1389202915666140528003249.
[22] V. Laengsri, U. Kerdpin, C. Plabplueng, L. Treeratanapiboon, P. Nuchnoi, Cervical cancer markers: epigenetics and microRNAs, Lab. Med. 49 (2018) 97–111, https://doi.org/10.1093/labmed/lmx080.
[23] A. Kozomara, M. Birgaoanu, S. Griffiths-Jones, miRBase: from microRNA sequences to function, Nucleic Acids Res. 47 (2019) D155–D162, https://doi.org/10.1093/nar/gky1141.
[24] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell. 116 (2004) 281–297, https://doi.org/10.1016/S0092-8674(04)00045-5.
[25] D.P. Bartel, Metazoan MicroRNAs, Cell. 173 (2018) 20–51, https://doi.org/10.1016/j.cell.2018.03.006.
[26] L.F.R. Gebert, I.J. MacRae, Regulation of microRNA function in animals, Nat. Rev. Mol. Cell Biol. (2018), https://doi.org/10.1038/s41580-018-0045-7.
[27] G.A. Cipolla, A non-canonical landscape of the microRNA system, Front. Genet. 5 (2014) 337, https://doi.org/10.3389/fgene.2014.00337.
[28] M. Fabbri, MicroRNAs and miRceptors: a new mechanism of action for intercellular communication, Philos. Trans. R. Soc. B Biol. Sci. 373 (2018) 20160486, https://doi.org/10.1098/rstb.2016.0486.
[29] L.F. Gulyaeva, N.E. Kushlinskiy, Regulatory mechanisms of microRNA expression, J. Transl. Med. 14 (2016) 143, https://doi.org/10.1186/s12967-016-0893-x.
[30] A.A. Svoronos, D.M. Engelman, F.J. Slack, OncomiR or tumor suppressor? The duplicity of MicroRNAs in cancer, Cancer Res. 76 (2016) 3666–3670, https://doi.org/10.1158/0008-5472.CAN-16-0359.
[31] K. Qian, T. Pietilä, M. Rönty, F. Michon, M.J. Frilander, J. Ritari, J. Tarkkanen, L. Paulín, P. Auvinen, E. Auvinen, Identification and validation of human papillomavirus encoded microRNAs, PLoS One 8 (2013) e70202, , https://doi.org/10.1371/journal.pone.0070202.
[32] E. Virtanen, T. Pietilä, P. Nieminen, K. Qian, E. Auvinen, Low expression levels of putative HPV encoded microRNAs in cervical samples, Springerplus. 5 (2016) 1856, https://doi.org/10.1186/s40064-016-3524-3.
[33] M.E. Harden, N. Prasad, A. Griffiths, K. Munger, Modulation of microRNA-mRNA target pairs by human papillomavirus 16 oncoproteins, MBio. 8 (2017), https://doi.org/10.1128/mBio.02170-16 (e02170–16).
[34] M.E. Harden, K. Munger, Perturbation of DROSHA and DICER expression by human papillomavirus 16 oncoproteins, Virology. 507 (2017) 192–198, https://doi.org/10.1016/j.virol.2017.04.022.
[35] K. Woods, J.M. Thomson, S.M. Hammond, Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors, J. Biol. Chem. 282 (2007) 2130–2134, https://doi.org/10.1074/jbc.C600252200.
[36] K.A. O'Donnell, E.A. Wentzel, K.I. Zeller, C.V. Dang, J.T. Mendell, c-Myc-regulated microRNAs modulate E2F1 expression, Nature 435 (2005) 839–843, https://doi.org/10.1038/nature03677.
[37] X. Liu, A. Dakic, R. Chen, G.L. Disbrow, Y. Zhang, Y. Dai, R. Schlegel, Cell-restricted immortalization by human papillomavirus correlates with telomerase activation and engagement of the hTERT promoter by Myc, J. Virol. 82 (2008) 11568–11576, https://doi.org/10.1128/JVI.01318-08.
[38] Y.-W. Wang, H.-S. Chang, C.-H. Lin, W.C.Y. Yu, HPV-18 E7 conjugates to c-Myc and mediates its transcriptional activity, Int. J. Biochem. Cell Biol. 39 (2007) 402–412, https://doi.org/10.1016/j.biocel.2006.09.006.
[39] X. Wang, H.-K. Wang, J.P. McCoy, N.S. Banerjee, J.S. Rader, T.R. Broker, C. Meyers, L.T. Chow, Z.-M. Zheng, Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6, RNA 15 (2009) 637–647, https://doi.org/10.1261/rna.1442309.
[40] M.K. Sannigrahi, R. Sharma, V. Singh, N.K. Panda, V. Rattan, M. Khullar, Role of host miRNA Hsa-miR-139-3p in HPV-16–induced carcinomas, Clin. Cancer Res. 23 (2017) 3884–3895, https://doi.org/10.1158/1078-0432.CCR-16-2936.
[41] W. Ben, Y. Yang, J. Yuan, J. Sun, M. Huang, D. Zhang, J. Zheng, Human papillomavirus 16 E6 modulates the expression of host microRNAs in cervical cancer, Taiwan. J. Obstet. Gynecol. 54 (2015) 364–370, https://doi.org/10.1016/j.tjog.2014.06.007.
[42] C.L. Au Yeung, T.Y. Tsang, P.L. Yau, T.T. Kwok, Human papillomavirus type 16 E6 induces cervical cancer cell migration through the p53/microRNA-23b/urokinase-type plasminogen activator pathway, Oncogene. 30 (2011) 2401–2410, https://doi.org/10.1038/onc.2010.613.
[43] C.L.A. Yeung, T.Y. Tsang, P.L. Yau, T.T. Kwok, Human papillomavirus type 16 E6 suppresses microRNA-23b expression in human cervical cancer cells through DNA methylation of the host gene C9orf3, Oncotarget 8 (2017) 12158–12173, https://doi.org/10.18632/oncotarget.14555.
[44] I. Martinez, A.S. Gardiner, K.F. Board, F.A. Monzon, R.P. Edwards, S.A. Khan, Human papillomavirus type 16 reduces the expression of microRNA-218 in

cervical carcinoma cells, Oncogene. 27 (2008) 2575–2582, https://doi.org/10.1038/sj.onc.1210919.
[45] N. Yamamoto, T. Kinoshita, N. Nohata, T. Itesako, H. Yoshino, H. Enokida, M. Nakagawa, M. Shozu, N. Seki, Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion by targeting focal adhesion pathways in cervical squamous cell carcinoma, Int. J. Oncol. 42 (2013) 1523–1532, https://doi.org/10.3892/ijo.2013.1851.
[46] W. Liu, G. Gao, X. Hu, Y. Wang, J.K. Schwarz, J.J. Chen, P.W. Grigsby, X. Wang, Activation of miR-9 by human papillomavirus in cervical cancer, Oncotarget 5 (2014) 11620–11630, https://doi.org/10.18632/oncotarget.2599.
[47] Y. Cheng, L. Geng, L. Zhao, P. Zuo, J. Wang, Human papillomavirus E6-regulated microRNA-20b promotes invasion in cervical cancer by targeting tissue inhibitor of metalloproteinase 2, Mol. Med. Rep. 16 (2017) 5464–5470, https://doi.org/10.3892/mmr.2017.7231.
[48] X. Liu, Up-regulation of miR-20a by HPV16 E6 exerts growth-promoting effects by targeting PDCD6 in cervical carcinoma cells, Biomed. Pharmacother. 102 (2018) 996–1002, https://doi.org/10.1016/j.biopha.2018.03.154.
[49] S. Hong, S. Cheng, W. Songock, J. Bodily, L.A. Laimins, Suppression of MicroRNA 424 Levels by human papillomaviruses is necessary for differentiation-dependent genome amplification, J. Virol. 91 (2017), https://doi.org/10.1128/JVI.01712-17.
[50] M. Melar-New, L.A. Laimins, Human papillomaviruses modulate expression of MicroRNA 203 upon epithelial differentiation to control levels of p63 proteins, J. Virol. 84 (2010) 5212–5221, https://doi.org/10.1128/JVI.00078-10.
[51] Q. Kong, W. Wang, P. Li, Regulator role of HPV E7 protein on miR-21 expression in cervical carcinoma cells and its functional implication, Int. J. Clin. Exp. Pathol. 8 (2015) 15808–15813 http://www.ncbi.nlm.nih.gov/pubmed/26884851 , Accessed date: 16 October 2018.
[52] F. Liu, S. Zhang, Z. Zhao, X. Mao, J. Huang, Z. Wu, L. Zheng, Q. Wang, MicroRNA-27b up-regulated by human papillomavirus 16 E7 promotes proliferation and suppresses apoptosis by targeting polo-like kinase2 in cervical cancer, Oncotarget 7 (2016) 19666–19679, https://doi.org/10.18632/oncotarget.7531.
[53] S. Zhang, F. Liu, X. Mao, J. Huang, J. Yang, X. Yin, L. Wu, L. Zheng, Q. Wang, Elevation of miR-27b by HPV16 E7 inhibits PPARγ expression and promotes proliferation and invasion in cervical carcinoma cells, Int. J. Oncol. 47 (2015) 1759–1766, https://doi.org/10.3892/ijo.2015.3162.
[54] D. Greco, N. Kivi, K. Qian, S.-K. Leivonen, P. Auvinen, E. Auvinen, Human papillomavirus 16 E5 modulates the expression of host MicroRNAs, PLoS One 6 (2011) e21646, , https://doi.org/10.1371/journal.pone.0021646.
[55] C. Liu, J. Lin, L. Li, Y. Zhang, W. Chen, Z. Cao, H. Zuo, C. Chen, K. Kee, HPV16 early gene E5 specifically reduces miRNA-196a in cervical cancer cells, Sci. Rep. 5 (2015) 7653, https://doi.org/10.1038/srep07653.
[56] H.M. Jung, B.L. Phillips, E.K. Chan, miR-375 activates p21 and suppresses telomerase activity by coordinately regulating HPV E6/E7, E6AP, CIP2A, and 14-3-3ζ, Mol. Cancer 13 (2014) 80, https://doi.org/10.1186/1476-4598-13-80.
[57] S. Wu, H. Chen, Anti-Condyloma acuminata mechanism of microRNAs-375 modulates HPV in cervical cancer cells via the UBE3A and IGF-1R pathway, Oncol. Lett. 16 (2018) 3241–3247, https://doi.org/10.3892/ol.2018.8983.
[58] G.J. Nuovo, X. Wu, S. Volinia, F. Yan, G. di Leva, N. Chin, A.F. Nicol, J. Jiang, G. Otterson, T.D. Schmittgen, C. Croce, Strong inverse correlation between microRNA-125b and human papillomavirus DNA in productive infection, Diagn. Mol. Pathol. 19 (2010) 135–143, https://doi.org/10.1097/PDM.0b013e3181c4daaa.
[59] J. Zhang, S. Li, Q. Yan, X. Chen, Y. Yang, X. Liu, X. Wan, Interferon-β induced microRNA-129-5p down-regulates HPV-18 E6 and E7 viral gene expression by targeting SP1 in cervical cancer cells, PLoS One 8 (2013) e81366, , https://doi.org/10.1371/journal.pone.0081366.
[60] C. Théry, L. Zitvogel, S. Amigorena, Exosomes: composition, biogenesis and function, Nat. Rev. Immunol. 2 (2002) 569–579, https://doi.org/10.1038/nri855.
[61] L. Barile, G. Vassalli, Exosomes: therapy delivery tools and biomarkers of diseases, Pharmacol. Ther. 174 (2017) 63–78, https://doi.org/10.1016/j.pharmthera.2017.02.020.
[62] S.E.L. Andaloussi, I. Mäger, X.O. Breakefield, M.J.A. Wood, Extracellular vesicles: biology and emerging therapeutic opportunities, Nat. Rev. Drug Discov. 12 (2013) 347–357, https://doi.org/10.1038/nrd3978.
[63] M.F. Baietti, Z. Zhang, E. Mortier, A. Melchior, G. Degeest, A. Geeraerts, Y. Ivarsson, F. Depoortere, C. Coomans, E. Vermeiren, P. Zimmermann, G. David, Syndecan–syntenin–ALIX regulates the biogenesis of exosomes, Nat. Cell Biol. 14 (2012) 677–685, https://doi.org/10.1038/ncb2502.
[64] M. Colombo, C. Moita, G. van Niel, J. Kowal, J. Vigneron, P. Benaroch, N. Manel, L.F. Moita, C. Théry, G. Raposo, Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles, J. Cell Sci. 126 (2013) 5553–5565, https://doi.org/10.1242/jcs.128868.
[65] B. Mateescu, E.J.K. Kowal, B.W.M. van Balkom, S. Bartel, S.N. Bhattacharyya, E.I. Buzás, A.H. Buck, P. de Candia, F.W.N. Chow, S. Das, T.A.P. Driedonks, L. Fernández-Messina, F. Haderk, A.F. Hill, J.C. Jones, K.R. Van Keuren-Jensen, C.P. Lai, C. Lässer, I. di Liegro, T.R. Lunavat, M.J. Lorenowicz, S.L.N. Maas, I. Mäger, M. Mittelbrunn, S. Momma, K. Mukherjee, M. Nawaz, D.M. Pegtel, M.W. Pfaffl, R.M. Schiffelers, H. Tahara, C. Théry, J.P. Tosar, M.H.M. Wauben, K.W. Witwer, E.N.M. Nolte-'t Hoen, Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper, J. Extracell. Vesicles. 6 (2017) 1286095, https://doi.org/10.1080/20013078.2017.1286095.
[66] M. Simons, G. Raposo, Exosomes – vesicular carriers for intercellular communication, Curr. Opin. Cell Biol. 21 (2009) 575–581, https://doi.org/10.1016/j.ceb.2009.03.007.
[67] D.J. Katzmann, C.J. Stefan, M. Babst, S.D. Emr, Vps27 recruits ESCRT machinery to endosomes during MVB sorting, J. Cell Biol. 162 (2003) 413–423, https://doi.org/10.1083/jcb.200302136.
[68] W.M. Henne, H. Stenmark, S.D. Emr, Molecular mechanisms of the membrane sculpting ESCRT pathway, Cold Spring Harb. Perspect. Biol. 5 (2013) 1–12, https://doi.org/10.1101/cshperspect.a016766.
[69] E.R. Abels, X.O. Breakefield, Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake, Cell. Mol. Neurobiol. 36 (2016) 301–312, https://doi.org/10.1007/s10571-016-0366-z.
[70] V.R. Minciacchi, M.R. Freeman, D. Di Vizio, Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes, Semin. Cell Dev. Biol. 40 (2015) 41–51, https://doi.org/10.1016/j.semcdb.2015.02.010.
[71] J.C. Akers, D. Gonda, R. Kim, B.S. Carter, C.C. Chen, Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies, J. Neuro-Oncol. 113 (2013) 1–11, https://doi.org/10.1007/s11060-013-1084-8.
[72] M.Y. Konoshenko, E.A. Lekchnov, A.V. Vlassov, P.P. Laktionov, Isolation of extracellular vesicles: general methodologies and latest trends, Biomed. Res. Int. 2018 (2018) 1–27, https://doi.org/10.1155/2018/8545347.
[73] J. McCullough, R.D. Fisher, F.G. Whitby, W.I. Sundquist, C.P. Hill, ALIX-CHMP4 interactions in the human ESCRT pathway, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 7687–7691, https://doi.org/10.1073/pnas.0801567105.
[74] C. Théry, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, A. Antoniou, T. Arab, F. Archer, G.K. Atkin-Smith, D.C. Ayre, J.-M. Bach, D. Bachurski, H. Baharvand, L. Balaj, S. Baldacchino, N.N. Bauer, A.A. Baxter, M. Bebawy, C. Beckham, A. Bedina Zavec, A. Benmoussa, A.C. Berardi, P. Bergese, E. Bielska, C. Blenkiron, S. Bobis-Wozowicz, E. Boilard, W. Boireau, A. Bongiovanni, F.E. Borràs, S. Bosch, C.M. Boulanger, X. Breakefield, A.M. Breglio, M.Á. Brennan, D.R. Brigstock, A. Brisson, M.L. Broekman, J.F. Bromberg, P. Bryl-Górecka, S. Buch, A.H. Buck, D. Burger, S. Busatto, D. Buschmann, B. Bussolati, E.I. Buzás, J.B. Byrd, G. Camussi, D.R. Carter, S. Caruso, L.W. Chamley, Y.-T. Chang, C. Chen, S. Chen, L. Cheng, A.R. Chin, A. Clayton, S.P. Clerici, A. Cocks, E. Cocucci, R.J. Coffey, A. Cordeiro-da-Silva, Y. Couch, F.A. Coumans, B. Coyle, R. Crescitelli, M.F. Criado, C. D'Souza-Schorey, S. Das, A. Datta Chaudhuri, P. de Candia, E.F. De Santana, O. De Wever, H.A. del Portillo, T. Demaret, S. Deville, A. Devitt, B. Dhondt, D. Di Vizio, L.C. Dieterich, V. Dolo, A.P. Dominguez Rubio, M. Dominici, M.R. Dourado, T.A. Driedonks, F.V. Duarte, H.M. Duncan, R.M. Eichenberger, K. Ekström, S.E.L. Andaloussi, C. Elie-Caille, U. Erdbrügger, J.M. Falcón-Pérez, F. Fatima, J.E. Fish, M. Flores-Bellver, A. Försönits, A. Frelet-Barrand, F. Fricke, G. Fuhrmann, S. Gabrielsson, A. Gámez-Valero, C. Gardiner, K. Gärtner, R. Gaudin, Y.S. Gho, B. Giebel, C. Gilbert, M. Gimona, I. Giusti, D.C. Goberdhan, A. Görgens, S.M. Gorski, D.W. Greening, J.C. Gross, A. Gualerzi, G.N. Gupta, D. Gustafson, A. Handberg, R.A. Haraszti, P. Harrison, H. Hegyesi, A. Hendrix, A.F. Hill, F.H. Hochberg, K.F. Hoffmann, B. Holder, H. Holthofer, B. Hosseinkhani, G. Hu, Y. Huang, V. Huber, S. Hunt, A.G.-E. Ibrahim, T. Ikezu, J.M. Inal, M. Isin, A. Ivanova, H.K. Jackson, S. Jacobsen, S.M. Jay, M. Jayachandran, G. Jenster, L. Jiang, S.M. Johnson, J.C. Jones, A. Jong, T. Jovanovic-Talisman, S. Jung, R. Kalluri, S. Kano, S. Kaur, Y. Kawamura, E.T. Keller, D. Khamari, E. Khomyakova, A. Khvorova, P. Kierulf, K.P. Kim, T. Kislinger, M. Klingeborn, D.J. Klinke, M. Kornek, M.M. Kosanović, Á.F. Kovács, E.-M. Krämer-Albers, S. Krasemann, M. Krause, I.V. Kurochkin, G.D. Kusuma, S. Kuypers, S. Laitinen, S.M. Langevin, L.R. Languino, J. Lannigan, C. Lässer, L.C. Laurent, G. Lavieu, E. Lázaro-Ibáñez, S. Le Lay, M.-S. Lee, Y.X.F. Lee, D.S. Lemos, M. Lenassi, A. Leszczynska, I.T. Li, K. Liao, S.F. Libregts, E. Ligeti, R. Lim, S.K. Lim, A. Linē, K. Linnemannstöns, A. Llorente, C.A. Lombard, M.J. Lorenowicz, Á.M. Lörincz, J. Lötvall, J. Lovett, M.C. Lowry, X. Loyer, Q. Lu, B. Lukomska, T.R. Lunavat, S.L. Maas, H. Malhi, A. Marcilla, J. Mariani, J. Mariscal, E.S. Martens-Uzunova, L. Martin-Jaular, M.C. Martinez, V.R. Martins, M. Mathieu, S. Mathivanan, M. Maugeri, L.K. McGinnis, M.J. McVey, D.G. Meckes, K.L. Meehan, I. Mertens, V.R. Minciacchi, A. Möller, M. Møller Jørgensen, A. Morales-Kastresana, J. Morhayim, F. Mullier, M. Muraca, L. Musante, V. Mussack, D.C. Muth, K.H. Myburgh, T. Najrana, M. Nawaz, I. Nazarenko, P. Nejsum, C. Neri, T. Neri, R. Nieuwland, L. Nimrichter, J.P. Nolan, E.N. Nolte-'t Hoen, N. Noren Hooten, L. O'Driscoll, T. O'Grady, A. O'Loghlen, T. Ochiya, M. Olivier, A. Ortiz, L.A. Ortiz, X. Osteikoetxea, O. Østergaard, M. Ostrowski, J. Park, D.M. Pegtel, H. Peinado, F. Perut, M.W. Pfaffl, D.G. Phinney, B.C. Pieters, R.C. Pink, D.S. Pisetsky, E. Pogge von Strandmann, I. Polakovicova, I.K. Poon, B.H. Powell, I. Prada, L. Pulliam, P. Quesenberry, A. Radeghieri, R.L. Raffai, S. Raimondo, J. Rak, M.I. Ramirez, G. Raposo, M.S. Rayyan, N. Regev-Rudzki, F.L. Ricklefs, P.D. Robbins, D.D. Roberts, S.C. Rodrigues, E. Rohde, S. Rome, K.M. Rouschop, A. Rughetti, A.E. Russell, P. Saá, S. Sahoo, E. Salas-Huenuleo, C. Sánchez, J.A. Saugstad, M.J. Saul, R.M. Schiffelers, R. Schneider, T.H. Schøyen, A. Scott, E. Shahaj, S. Sharma, O. Shatnyeva, F. Shekari, G.V. Shelke, A.K. Shetty, K. Shiba, P.R.-M. Siljander, A.M. Silva, A. Skowronek, O.L. Snyder, R.P. Soares, B.W. Sódar, C. Soekmadji, J. Sotillo, P.D. Stahl, W. Stoorvogel, S.L. Stott, E.F. Strasser, S. Swift, H. Tahara, M. Tewari, K. Timms, S. Tiwari, R. Tixeira, M. Tkach, W.S. Toh, R. Tomasini, A.C. Torrecilhas, J.P. Tosar, V. Toxavidis, L. Urbanelli, P. Vader, B.W. van Balkom, S.G. van der Grein, J. Van Deun, M.J. van Herwijnen, K. Van Keuren-Jensen, G. van Niel, M.E. van Royen, A.J. van Wijnen, M.H. Vasconcelos, I.J. Vechetti, T.D. Veit, L.J. Vella, É. Velot, F.J. Verweij, B. Vestad, J.L. Viñas, T. Visnovitz, K.V. Vukman, J. Wahlgren, D.C. Watson, M.H. Wauben, A. Weaver, J.P. Webber, V. Weber, A.M. Wehman, D.J. Weiss, J.A. Welsh, S. Wendt, A.M. Wheelock, Z. Wiener, L. Witte, J. Wolfram, A. Xagorari, P. Xander, J. Xu, X. Yan, M. Yáñez-Mó, H. Yin, Y. Yuana, V. Zappulli, J. Zarubova, V. Žėkas, J. Zhang, Z. Zhao, L. Zheng, A.R. Zheutlin, A.M. Zickler, P. Zimmermann, A.M. Zivkovic, D. Zocco, E.K. Zuba-Surma, Minimal information for studies of

extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J. Extracell. Vesicles 7 (2018) 1535750, https://doi.org/10.1080/20013078.2018.1535750.
[75] N. Kosaka, H. Iguchi, K. Hagiwara, Y. Yoshioka, F. Takeshita, T. Ochiya, Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis, J. Biol. Chem. 288 (2013) 10849–10859, https://doi.org/10.1074/jbc.M112.446831.
[76] C. Villarroya-Beltri, C. Gutiérrez-Vázquez, F. Sánchez-Cabo, D. Pérez-Hernández, J. Vázquez, N. Martin-Cofreces, D.J. Martinez-Herrera, A. Pascual-Montano, M. Mittelbrunn, F. Sánchez-Madrid, Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs, Nat. Commun. 4 (2013) 2980, https://doi.org/10.1038/ncomms3980.
[77] D. Koppers-Lalic, M. Hackenberg, I.V. Bijnsdorp, M.A.J. van Eijndhoven, P. Sadek, D. Sie, N. Zini, J.M. Middeldorp, B. Ylstra, R.X. de Menezes, T. Würdinger, G.A. Meijer, D.M. Pegtel, Nontemplated nucleotide additions distinguish the small RNA composition in cells from Exosomes, Cell Rep. 8 (2014) 1649–1658, https://doi.org/10.1016/J.CELREP.2014.08.027.
[78] J. Zhang, S. Li, L. Li, M. Li, C. Guo, J. Yao, S. Mi, Exosome and exosomal MicroRNA: trafficking, sorting, and function, Genomics. Proteomics Bioinformatics. 13 (2015) 17–24, https://doi.org/10.1016/J.GPB.2015.02.001.
[79] A. Iavello, V.S.L. Frech, C. Gai, M.C. Deregibus, P.J. Quesenberry, G. Camussi, Role of Alix in miRNA packaging during extracellular vesicle biogenesis, Int. J. Mol. Med. 37 (2016) 958–966, https://doi.org/10.3892/ijmm.2016.2488.
[80] S.L.N. Maas, X.O. Breakefield, A.M. Weaver, Extracellular Vesicles: Unique Intercellular Delivery Vehicles, Trends Cell Biol 27 (2017) 172–188, https://doi.org/10.1016/j.tcb.2016.11.003.
[81] N. Iraci, T. Leonardi, F. Gessler, B. Vega, S. Pluchino, Focus on Extracellular Vesicles: Physiological Role and Signalling Properties of Extracellular Membrane Vesicles, Int. J. Mol. Sci. 17 (2016) 171, https://doi.org/10.3390/ijms17020171.
[82] K.C. French, M.A. Antonyak, R.A. Cerione, Extracellular vesicle docking at the cellular port: Extracellular vesicle binding and uptake, Semin. Cell Dev. Biol. 67 (2017) 48–55, https://doi.org/10.1016/j.semcdb.2017.01.002.
[83] K. Wu, F. Xing, S.-Y. Wu, K. Watabe, Extracellular vesicles as emerging targets in cancer: Recent development from bench to bedside, Biochim. Biophys. Acta - Rev. Cancer. 1868 (2017) 538–563, https://doi.org/10.1016/j.bbcan.2017.10.001.
[84] R. Xu, A. Rai, M. Chen, W. Suwakulsiri, D.W. Greening, R.J. Simpson, Extracellular vesicles in cancer — implications for future improvements in cancer care, Nat. Rev. Clin. Oncol. 15 (2018) 617–638, https://doi.org/10.1038/s41571-018-0036-9.
[85] A.E. Sedgwick, C. D'Souza-Schorey, The biology of extracellular microvesicles, Traffic. 19 (2018) 319–327, https://doi.org/10.1111/tra.12558.
[86] C. Roma-Rodrigues, A.R. Fernandes, P.V. Baptista, Exosome in tumour microenvironment: overview of the crosstalk between normal and cancer cells, Biomed. Res. Int. 2014 (2014) 179486, https://doi.org/10.1155/2014/179486.
[87] D. Guenat, F. Hermetet, J.-L. Prétet, C. Mougin, Exosomes and other extracellular vesicles in HPV transmission and carcinogenesis, Viruses. 9 (2017) 211, https://doi.org/10.3390/v9080211.
[88] K. Al-Nedawi, B. Meehan, J. Micallef, V. Lhotak, L. May, A. Guha, J. Rak, Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells, Nat. Cell Biol. 10 (2008) 619–624, https://doi.org/10.1038/ncb1725.
[89] M. Demory Beckler, J.N. Higginbotham, J.L. Franklin, A.-J. Ham, P.J. Halvey, I.E. Imasuen, C. Whitwell, M. Li, D.C. Liebler, R.J. Coffey, Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS, Mol. Cell. Proteomics 12 (2013) 343–355, https://doi.org/10.1074/mcp.M112.022806.
[90] A. Hoshino, B. Costa-Silva, T.-L. Shen, G. Rodrigues, A. Hashimoto, M. Tesic Mark, H. Molina, S. Kohsaka, A. Di Giannatale, S. Ceder, S. Singh, C. Williams, N. Soplop, K. Uryu, L. Pharmer, T. King, L. Bojmar, A.E. Davies, Y. Ararso, T. Zhang, H. Zhang, J. Hernandez, J.M. Weiss, V.D. Dumont-Cole, K. Kramer, L.H. Wexler, A. Narendran, G.K. Schwartz, J.H. Healey, P. Sandstrom, K. Jørgen Labori, E.H. Kure, P.M. Grandgenett, M.A. Hollingsworth, M. de Sousa, S. Kaur, M. Jain, K. Mallya, S.K. Batra, W.R. Jarnagin, M.S. Brady, O. Fodstad, V. Muller, K. Pantel, A.J. Minn, M.J. Bissell, B.A. Garcia, Y. Kang, V.K. Rajasekhar, C.M. Ghajar, I. Matei, H. Peinado, J. Bromberg, D. Lyden, Tumour exosome integrins determine organotropic metastasis, Nature 527 (2015) 329–335, https://doi.org/10.1038/nature15756.
[91] H. Peinado, S. Lavotshkin, D. Lyden, The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts, Semin. Cancer Biol. 21 (2011) 139–146, https://doi.org/10.1016/j.semcancer.2011.01.002.
[92] Z. Qian, Q. Shen, X. Yang, Y. Qiu, W. Zhang, The role of extracellular vesicles: an epigenetic view of the Cancer microenvironment, Biomed. Res. Int. 2015 (2015) 1–8, https://doi.org/10.1155/2015/649161.
[93] M. Ono, N. Kosaka, N. Tominaga, Y. Yoshioka, F. Takeshita, R.-U. Takahashi, M. Yoshida, H. Tsuda, K. Tamura, T. Ochiya, Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells, Sci. Signal 7 (2014), https://doi.org/10.1126/scisignal.2005231 ra63-ra63.
[94] N. Kosaka, Y. Yoshioka, Y. Fujita, T. Ochiya, Versatile roles of extracellular vesicles in cancer, J. Clin. Invest. 126 (2016) 1163–1172, https://doi.org/10.1172/JCI81130.
[95] J. Wolfers, A. Lozier, G. Raposo, A. Regnault, C. Théry, C. Masurier, C. Flament, S. Pouzieux, F. Faure, T. Tursz, E. Angevin, S. Amigorena, L. Zitvogel, Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming, Nat. Med. 7 (2001) 297–303, https://doi.org/10.1038/85438.
[96] M. Alenquer, M. Amorim, Exosome biogenesis, regulation, and function in viral infection, Viruses. 7 (2015) 5066–5083, https://doi.org/10.3390/v7092862.
[97] M.R. Anderson, F. Kashanchi, S. Jacobson, Exosomes in viral disease, Neurotherapeutics. 13 (2016) 535–546, https://doi.org/10.1007/s13311-016-0450-6.
[98] A. Honegger, D. Schilling, S. Bastian, J. Sponagel, V. Kuryshev, H. Sültmann, M. Scheffner, K. Hoppe-Seyler, F. Hoppe-Seyler, Dependence of intracellular and exosomal microRNAs on viral E6/E7 oncogene expression in HPV-positive tumor cells, PLoS Pathog. 11 (2015) e1004712, , https://doi.org/10.1371/journal.ppat.1004712.
[99] E. Hernando, microRNAs and cancer: role in tumorigenesis, patient classification and therapy, Clin. Transl. Oncol. 9 (2007) 155–160.
[100] W.-O. Lui, N. Pourmand, B.K. Patterson, A. Fire, Patterns of known and novel small RNAs in human cervical cancer, Cancer Res. 67 (2007) 6031–6043, https://doi.org/10.1158/0008-5472.CAN-06-0561.
[101] B. Muralidhar, L. Goldstein, G. Ng, D. Winder, R. Palmer, E. Gooding, N. Barbosa-Morais, G. Mukherjee, N. Thorne, I. Roberts, M. Pett, N. Coleman, Global microRNA profiles in cervical squamous cell carcinoma depend on Drosha expression levels, J. Pathol. 212 (2007) 368–377, https://doi.org/10.1002/path.2179.
[102] X. Wang, S. Tang, S.-Y. Le, R. Lu, J.S. Rader, C. Meyers, Z.-M. Zheng, Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth, PLoS One 3 (2008) e2557, , https://doi.org/10.1371/journal.pone.0002557.
[103] P.M. Pereira, J.P. Marques, A.R. Soares, L. Carreto, M.A.S. Santos, MicroRNA expression variability in human cervical tissues, PLoS One 5 (2010) e11780, , https://doi.org/10.1371/journal.pone.0011780.
[104] S.M. Wilting, P.J.F. Snijders, W. Verlaat, A. Jaspers, M.A. van de Wiel, W.N. van Wieringen, G.A. Meijer, G.G. Kenter, Y. Yi, C. le Sage, R. Agami, C.J.L.M. Meijer, R.D.M. Steenbergen, Altered microRNA expression associated with chromosomal changes contributes to cervical carcinogenesis, Oncogene. 32 (2013) 106–116, https://doi.org/10.1038/onc.2012.20.
[105] W. Mo, C. Tong, Y. Zhang, H. Lu, microRNAs' differential regulations mediate the progress of Human Papillomavirus (HPV)-induced Cervical Intraepithelial Neoplasia (CIN), BMC Syst. Biol. 9 (2015) 4, https://doi.org/10.1186/s12918-015-0145-3.
[106] V. González-Quintana, L. Palma-Berré, A.D. Campos-Parra, E. López-Urrutia, O. Peralta-Zaragoza, R. Vazquez-Romo, C. Pérez-Plasencia, MicroRNAs are involved in cervical cancer development, progression, clinical outcome and improvement treatment response (review), Oncol. Rep. 35 (2016) 3–12, https://doi.org/10.3892/or.2015.4369.
[107] B. Pardini, D. De Maria, A. Francavilla, C. Di Gaetano, G. Ronco, A. Naccarati, MicroRNAs as markers of progression in cervical cancer: a systematic review, BMC Cancer 18 (2018) 696, https://doi.org/10.1186/s12885-018-4590-4.
[108] Y. Li, F. Wang, J. Xu, F. Ye, Y. Shen, J. Zhou, W. Lu, X. Wan, D. Ma, X. Xie, Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV-related target genes for miR-29, J. Pathol. 224 (2011) 484–495, https://doi.org/10.1002/path.2873.
[109] C.B. Lajer, E. Garnæs, L. Friis-Hansen, B. Norrild, M.H. Therkildsen, M. Glud, M. Rossing, H. Lajer, D. Svane, L. Skotte, L. Specht, C. Buchwald, F.C. Nielsen, The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer, Br. J. Cancer 106 (2012) 1526–1534, https://doi.org/10.1038/bjc.2012.109.
[110] J.-W. Lee, C.H. Choi, J.-J. Choi, Y.-A. Park, S.-J. Kim, S.Y. Hwang, W.Y. Kim, T.-J. Kim, J.-H. Lee, B.-G. Kim, D.-S. Bae, Altered MicroRNA expression in cervical carcinomas, Clin. Cancer Res. 14 (2008) 2535–2542, https://doi.org/10.1158/1078-0432.CCR-07-1231.
[111] L. Liu, X. Yu, X. Guo, Z. Tian, M. Su, Y. Long, C. Huang, F. Zhou, M. Liu, X. Wu, X. Wang, MiR-143 is downregulated in cervical cancer and promotes apoptosis and inhibits tumor formation by targeting Bcl-2, Mol. Med. Rep. 5 (2012) 753–760, https://doi.org/10.3892/mmr.2011.696.
[112] D. Witten, R. Tibshirani, S.G. Gu, A. Fire, W.-O. Lui, Ultra-high throughput sequencing-based small RNA discovery and discrete statistical biomarker analysis in a collection of cervical tumours and matched controls, BMC Biol. 8 (2010) 58, https://doi.org/10.1186/1741-7007-8-58.
[113] T. Cheung, K.M. Man, M. Yu, S. Yim, N.S.S. Siu, K.W.K. Lo, G. Doran, R.R.Y. Wong, V.W. Wang, D.I. Smith, M.J. Worley Jr., R.S. Berkowitz, T.K.H. Chung, Y. Wong, Dysregulated microRNAs in the pathogenesis and progression of cervical neoplasm, Cell Cycle 11 (2012) 2876–2884, https://doi.org/10.4161/cc.21278.
[114] X. Zhu, K. Er, C. Mao, Q. Yan, H. Xu, Y. Zhang, J. Zhu, F. Cui, W. Zhao, H. Shi, miR-203 suppresses tumor growth and angiogenesis by targeting VEGFA in cervical cancer, Cell. Physiol. Biochem. 32 (2013) 64–73, https://doi.org/10.1159/000350125.
[115] J. Chen, D. Yao, Y. Li, H. Chen, C. He, N. Ding, Y. Lu, T. Ou, S. Zhao, L. Li, F. Long, Serum microRNA expression levels can predict lymph node metastasis in patients with early-stage cervical squamous cell carcinoma, Int. J. Mol. Med. 32 (2013) 557–567, https://doi.org/10.3892/ijmm.2013.1424.
[116] K. Gocze, K. Gombos, K. Kovacs, K. Juhasz, P. Gocze, I. Kiss, MicroRNA expressions in HPV-induced cervical dysplasia and cancer, Anticancer Res. 35 (2015) 523–530 http://www.ncbi.nlm.nih.gov/pubmed/25550598 , Accessed date: 23 July 2018.
[117] X. Wang, H.-K. Wang, Y. Li, M. Hafner, N.S. Banerjee, S. Tang, D. Briskin, C. Meyers, L.T. Chow, X. Xie, T. Tuschl, Z.-M. Zheng, microRNAs are biomarkers of oncogenic human papillomavirus infections, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 4262–4267, https://doi.org/10.1073/pnas.1401430111.
[118] K. Zeng, W. Zheng, X. Mo, F. Liu, M. Li, Z. Liu, W. Zhang, X. Hu, Dysregulated

microRNAs involved in the progression of cervical neoplasm, Arch. Gynecol. Obstet. 292 (2015) 905–913, https://doi.org/10.1007/s00404-015-3702-5.
[119] C.H. Lawrie, S. Gal, H.M. Dunlop, B. Pushkaran, A.P. Liggins, K. Pulford, A.H. Banham, F. Pezzella, J. Boultwood, J.S. Wainscoat, C.S.R. Hatton, A.L. Harris, Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma, Br. J. Haematol. 141 (2008) 672–675, https://doi.org/10.1111/j.1365-2141.2008.07077.x.
[120] P.S. Mitchell, R.K. Parkin, E.M. Kroh, B.R. Fritz, S.K. Wyman, E.L. Pogosova-Agadjanyan, A. Peterson, J. Noteboom, K.C. O'Briant, A. Allen, D.W. Lin, N. Urban, C.W. Drescher, B.S. Knudsen, D.L. Stirewalt, R. Gentleman, R.L. Vessella, P.S. Nelson, D.B. Martin, M. Tewari, Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 10513–10518, https://doi.org/10.1073/pnas.0804549105.
[121] X. Chen, Y. Ba, L. Ma, X. Cai, Y. Yin, K. Wang, J. Guo, Y. Zhang, J. Chen, X. Guo, Q. Li, X. Li, W. Wang, Y. Zhang, J. Wang, X. Jiang, Y. Xiang, C. Xu, P. Zheng, J. Zhang, R. Li, H. Zhang, X. Shang, T. Gong, G. Ning, J. Wang, K. Zen, J. Zhang, C.-Y. Zhang, Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases, Cell Res. 18 (2008) 997–1006, https://doi.org/10.1038/cr.2008.282.
[122] A. Turchinovich, L. Weiz, A. Langheinz, B. Burwinkel, Characterization of extracellular circulating microRNA, Nucleic Acids Res. 39 (2011) 7223–7233, https://doi.org/10.1093/nar/gkr254.
[123] P. Landry, I. Plante, D.L. Ouellet, M.P. Perron, G. Rousseau, P. Provost, Existence of a microRNA pathway in anucleate platelets, Nat. Struct. Mol. Biol. 16 (2009) 961–966, https://doi.org/10.1038/nsmb.1651.
[124] P. Willeit, A. Zampetaki, K. Dudek, D. Kaudewitz, A. King, N.S. Kirkby, R. Crosby-Nwaobi, M. Prokopi, I. Drozdov, S.R. Langley, S. Sivaprasad, H.S. Markus, J.A. Mitchell, T.D. Warner, S. Kiechl, M. Mayr, Circulating MicroRNAs as novel biomarkers for platelet activation, Circ. Res. 112 (2013) 595–600, https://doi.org/10.1161/CIRCRESAHA.111.300539.
[125] L. Zavesky, E. Jandakova, R. Turyna, L. Langmeierova, V. Weinberger, L. Minar, M. Kohoutova, New perspectives in diagnosis of gynaecological cancers: emerging role of circulating microRNAs as novel biomarkers, Neoplasma. 62 (2015) 509–520, https://doi.org/10.4149/neo_2015_062.
[126] J. Yu, Y. Wang, R. Dong, X. Huang, S. Ding, H. Qiu, Circulating microRNA-218 was reduced in cervical cancer and correlated with tumor invasion, J. Cancer Res. Clin. Oncol. 138 (2012) 671–674, https://doi.org/10.1007/s00432-012-1147-9.
[127] S. Zhao, D. Yao, J. Chen, N. Ding, Circulating *miRNA-20a* and *miRNA-203* for screening lymph node metastasis in early stage cervical Cancer, Genet. Test. Mol. Biomarkers. 17 (2013) 631–636, https://doi.org/10.1089/gtmb.2013.0085.
[128] Q. Ma, G. Wan, S. Wang, W. Yang, J. Zhang, X. Yao, Serum microRNA-205 as a novel biomarker for cervical cancer patients, Cancer Cell Int. 14 (2014) 81, https://doi.org/10.1186/s12935-014-0081-0.
[129] W. Jia, Y. Wu, Q. Zhang, G. Gao, C. Zhang, Y. Xiang, Expression profile of circulating microRNAs as a promising fingerprint for cervical cancer diagnosis and monitoring, Mol. Clin. Oncol. 3 (2015) 851–858, https://doi.org/10.3892/mco.2015.560.
[130] P. Liu, F. Xin, C.F. Ma, Clinical significance of serum miR-196a in cervical intraepithelial neoplasia and cervical cancer, Genet. Mol. Res. 14 (2015) 17995–18002, https://doi.org/10.4238/2015.December.22.25.
[131] Y. Zhang, D. Zhang, F. Wang, D. Xu, Y. Guo, W. Cui, Serum miRNAs panel (miR-16-2*, miR-195, miR-2861, miR-497) as novel non-invasive biomarkers for detection of cervical cancer, Sci. Rep. 5 (2015) 17942, https://doi.org/10.1038/srep17942.
[132] Y. Nagamitsu, H. Nishi, T. Sasaki, Y. Takaesu, F. Terauchi, K. Isaka, Profiling analysis of circulating microRNA expression in cervical cancer, Mol. Clin. Oncol. 5 (2016) 189–194, https://doi.org/10.3892/mco.2016.875.
[133] F. Xin, P. Liu, C.-F. Ma, A circulating serum miRNA panel as early detection biomarkers of cervical intraepithelial neoplasia, Eur. Rev. Med. Pharmacol. Sci. 20 (2016) 4846–4851 http://www.ncbi.nlm.nih.gov/pubmed/27981553 , Accessed date: 8 August 2018.
[134] L. Sun, R. Jiang, J. Li, B. Wang, C. Ma, Y. Lv, N. Mu, MicoRNA-425-5p is a potential prognostic biomarker for cervical cancer, Ann. Clin. Biochem. 54 (2017) 127–133, https://doi.org/10.1177/0004563216649377.
[135] C. Li, X. Zheng, W. Li, F. Bai, J. Lyu, Q.H. Meng, Serum miR-486-5p as a diagnostic marker in cervical cancer: with investigation of potential mechanisms, BMC Cancer 18 (2018) 61, https://doi.org/10.1186/s12885-017-3753-z.
[136] S. Kawai, T. Fujii, I. Kukimoto, H. Yamada, N. Yamamoto, M. Kuroda, S. Otani, R. Ichikawa, E. Nishio, Y. Torii, A. Iwata, Identification of miRNAs in cervical mucus as a novel diagnostic marker for cervical neoplasia, Sci. Rep. 8 (2018) 7070, https://doi.org/10.1038/s41598-018-25310-1.
[137] W.-T. Wang, Y.-N. Zhao, J.-X. Yan, M.-Y. Weng, Y. Wang, Y.-Q. Chen, S.-J. Hong, Differentially expressed microRNAs in the serum of cervical squamous cell carcinoma patients before and after surgery, J. Hematol. Oncol. 7 (2014) 6, https://doi.org/10.1186/1756-8722-7-6.
[138] Q. Kong, Z. Tang, F. Xiang, J. Jiang, H. Yue, R. Wu, X. Kang, Diagnostic Value of Serum hsa-mir-92a in Patients with Cervical Cancer, Clin. Lab. 63 (2017) 335–340 http://www.ncbi.nlm.nih.gov/pubmed/28182350 , Accessed date: 4 September 2018.
[139] W. Jiang, J.J. Pan, Y.H. Deng, M.R. Liang, L.H. Yao, Down-regulated serum microRNA-101 is associated with aggressive progression and poor prognosis of cervical cancer, J. Gynecol. Oncol. 28 (2017) e75, , https://doi.org/10.3802/jgo.2017.28.e75.
[140] W. You, Y. Wang, J. Zheng, Plasma miR-127 and miR-218 might serve as potential biomarkers for cervical cancer, Reprod. Sci. 22 (2015) 1037–1041, https://doi.org/10.1177/1933719115570902.
[141] B.-B. Tang, S.-Y. Liu, Y. Zhan, L.-Q. Wei, X.-L. Mao, J. Wang, L. Li, Z.-X. Lu, microRNA-218 expression and its association with the clinicopathological characteristics of patients with cervical cancer, Exp. Ther. Med. 10 (2015) 269–274, https://doi.org/10.3892/etm.2015.2455.
[142] T. Lopatina, C. Gai, M.C. Deregibus, S. Kholia, G. Camussi, Cross talk between cancer and mesenchymal stem cells through extracellular vesicles carrying nucleic acids, Front. Oncol. 6 (2016) 125, https://doi.org/10.3389/fonc.2016.00125.
[143] X. Zhang, X. Yuan, H. Shi, L. Wu, H. Qian, W. Xu, Exosomes in cancer: small particle, big player, J. Hematol. Oncol. 8 (2015) 83, https://doi.org/10.1186/s13045-015-0181-x.
[144] J. Kaiser, Malignant messengers, Science 352 (2016) 164–166, https://doi.org/10.1126/science.352.6282.164 (80-.).
[145] J. Liu, H. Sun, X. Wang, Q. Yu, S. Li, X. Yu, W. Gong, Increased exosomal MicroRNA-21 and MicroRNA-146a levels in the cervicovaginal lavage specimens of patients with cervical cancer, Int. J. Mol. Sci. 15 (2014) 758–773, https://doi.org/10.3390/ijms15010758.
[146] A. Honegger, J. Leitz, J. Bulkescher, K. Hoppe-Seyler, F. Hoppe-Seyler, Silencing of human papillomavirus (HPV) *E6/E7* oncogene expression affects both the contents and the amounts of extracellular microvesicles released from HPV-positive cancer cells, Int. J. Cancer 133 (2013) 1631–1642, https://doi.org/10.1002/ijc.28164.
[147] M.V. Chiantore, G. Mangino, M. Iuliano, M.S. Zangrillo, I. De Lillis, G. Vaccari, R. Accardi, M. Tommasino, S. Columba Cabezas, M. Federico, G. Fiorucci, G. Romeo, Human papillomavirus E6 and E7 oncoproteins affect the expression of cancer-related microRNAs: additional evidence in HPV-induced tumorigenesis, J. Cancer Res. Clin. Oncol. 142 (2016) 1751–1763, https://doi.org/10.1007/s00432-016-2189-1.
[148] M.E. Harden, K. Munger, Human papillomavirus 16 E6 and E7 oncoprotein expression alters microRNA expression in extracellular vesicles, Virology. 508 (2017) 63–69, https://doi.org/10.1016/j.virol.2017.05.005.
[149] A. Honegger, D. Schilling, H. Sültmann, K. Hoppe-Seyler, F. Hoppe-Seyler, Identification of E6/E7-dependent MicroRNAs in HPV-positive cancer cells, Methods in Molecular Biology, 2018, pp. 119–134, , https://doi.org/10.1007/978-1-4939-7435-1_10.
[150] R. Mayeux, Biomarkers: potential uses and limitations, NeuroRx. 1 (2004) 182–188, https://doi.org/10.1602/neurorx.1.2.182.
[151] S. Kumar, A. Mohan, R. Guleria, Biomarkers in cancer screening, research and detection: present and future: a review, Biomarkers. 11 (2006) 385–405, https://doi.org/10.1080/13547500600775011.
[152] R. Kumarswamy, I. Volkmann, T. Thum, Regulation and function of miRNA-21 in health and disease, RNA Biol. 8 (2011) 706–713, https://doi.org/10.4161/rna.8.5.16154.