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Mini-review

MiRNA in melanoma-derived exosomes

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ABSTRACT

Proteins, RNAs and viruses can be spread through exosomes, therefore transport utilizing these nanovesicles is of the great interest. MiRNAs are common exosomal constituents capable of influencing expression of a variety of target genes. MiRNA signatures of exosomes are unique in cancer patients and differ from those in normal controls. The knowledge about miRNA profiles of tumor-derived exosomes may contribute to better diagnosis, determination of tumor progression and response to treatment, as well as to the development of targeted therapies. We summarize the current knowledge with regard to miRNAs that are found in exosomes derived from tumors, particularly from melanoma.

1. Short characterization of biogenesis, structure and composition of exosomes

Exosomes are small, intraluminal vesicles (30–150 nm in diameter), that were first described by Trams and coworkers [1]. Many cell types release exosomes, including reticulocytes, B and T cells, dendritic cells, mast cells and epithelial cells, as well as tumor cells [2–4] Exosomes have been detected in most body fluids [5,6]. They are composed of a lipid bilayer membrane containing ceramides, cholesterol, sphingolipids and phosphoglycerides [7–9]. Exosomes are enriched with a number of proteins, including members of the tetraspanin family (CD9, CD81, CD82, CD63) and heat shock proteins (Hsp60, Hsp70, Hsp90), proteins participating in the biogenesis of the multivesicular bodies (annexins, Rab family GTPases, and ESCRT complex proteins) as well as interleukins and components of certain signaling pathways e.g. Wnt-β-catenin signaling proteins [10–13]. Several proteins present in exosomes are specific for the donor cells. Melanoma-derived exosomes contain an enhanced level of Melan A [14]. It is assumed that the lipoprotein content of exosomal membranes ensures the exosomal stability in the extracellular environment [15,16] and the ability to adhere to the target cells [17]. Exosomal biogenesis takes place in an endosomal compartment, called the multivesicular bodies (MVBs). Cytoplasmic RNA molecules and proteins are selectively packed into exosomes. Profiling of mRNA revealed that several hundred transcripts were enriched in exosomes in comparison to donor cancer cells [19]. Similarly, the miRNA profiles substantially differed between exosomes and cancer cells [18,20]. Recently, two short sequence motifs were identified which function as exosomal packaging signals for miRNAs, whereas three other motifs were identified which were not found in miRNAs from exosomes but in those retained inside the cell [21]. The exosomal packaging signals used to control miRNA sorting into exosomes were specifically recognized by sumoylated heterogeneous nuclear ribonucleoproteins, mainly hnRNPA2B1 and hnRNPA1.

Exosomes are released into the extracellular space by exocytic fusion with the plasma membrane [22–26]. Their secretion is considered to be a highly organized and controlled process stimulated by various chemical, biological or mechanical factors. It has been

Abbreviations: AGO-2, argonaute protein 2; BMDCs, bone marrow-derived cells; BMP4, bone morphogenetic protein 4; CCND1, cyclin D1; CDK4, cyclin-dependent kinase; DGCR8, DiGeorge critical region gene 8; EMT, epithelial to mesenchymal transition; ESCRT, endosomal sorting complexes required for transport; EZH2, histone-lysine N-methyltransferase; Hsp, heat shock protein; HMC-1, human mast cell line; JAK-STAT, Janus kinase, signal transducer and activator of transcription; KCNMA1, calcium ion-regulated potassium channel protein; MET, mesenchymal to epithelial transition; MITF, microphthalmia-associated transcription factor; MSCs, mesenchymal stem cells; MVBs, multivesicular bodies; MYLIP, myosin regulatory light chain-interacting protein; NHEMs, neonatal human epidermal melanocytes; NIK, NF-kappaB-inducing kinase; PTEN, phosphatase and tensin homolog; RBP1-like, retinoblastoma binding protein 1 like; RISC, RNA-induced silencing complex; RUNX3, runt-related transcription factor 3; TD-exosomes, tumor derived exosomes; TLR, Toll-like receptor; Tregs, regulatory T cells.

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demonstrated that heparanase, an enzyme up-regulated in many tumor cell lines is involved in the secretion of exosomes [27]. The exosomal secretion could be induced by γ-irradiation followed by DNA damage [28], or by treatment with statins or calcium ionophores [8,29]. It has also been shown that mechanical detachment of breast cancer cells from various surfaces increases the exosomal secretion [30]. Moreover, low pH [31] or hypoxic conditions in cultures of melanoma or breast cancer cell lines, respectively, leads to a significant enhancement of exosomal release [32]. Secreted exosomes are taken up by the target cells through three possible mechanisms. The first one comprises simple fusion with the cellular membrane, directly releasing the content of the vesicles into the cytoplasm [17,33]. The second one assumes exosomal uptake by endocytosis [5,34]. In the third one, the uptake is a non-random process, but it is dependent on the presence of distinct receptor proteins that enable the binding of exosomes to the target cells [35]. A general scheme of exosomal biogenesis, the release from the cell of origin as well mechanisms of exosomal uptake by the target cell are presented in Fig. 1 and more information can be found in several recently published review articles [35–39]. Exosomes were originally thought to play a main role in cellular debris disposal, but nowadays their role in long distance cell–cell communication is increasingly acknowledged as they function as important transporters of miRNAs, piRNAs, lncRNAs, rRNAs, snRNA, snoRNAs, tRNAs, mRNAs, DNA fragments, and proteins [40,41]. According to the Exocarta database (http://www.exocarta.org) which gathers information about the contents of exosomes, 4563 proteins, 1639 mRNAs, 764 miRNAs and 194 lipids have already been identified in exosomes from different cell types of multiple organisms.

2. Exosomes as miRNA carrying vesicles

Exosomal transfer of mRNAs and miRNAs has been recognized as an important cellular communication system for the exchange of genetic and epigenetic information between cells [5,40,42]. MiRNAs (microRNAs) are small (19–25 nt), non-coding regulatory RNAs. The miRBase database which contains information for miRNAs (release 20, http://www.mirbase.org) lists 24521 entries representing hairpin precursor miRNAs, and 30424 mature miRNA products from 206 species. Among them are 1872 miRNAs from *Homo sapiens*.

MiRNA biogenesis (Fig. 2) begins with their transcription by RNA polymerase II generating primary miRNAs (pri-miRNA) comprising a hairpin stem, a terminal loop and two single stranded regions. Pri-miRNAs are processed in the nucleus by DROSHA and DGCR8 (DiGeorge Critical Region gene 8) that is responsible for the precise pri-miRNA cleavage. This process generates short hairpin structures, the pre-miRNAs, which are exported from the nucleus by exportin-5. The maturation of pre-miRNAs occurs in the cytoplasm through cleavage by DICER. The mature miRNA is then incorporated into a protein complex called RISC (RNA induced silencing complex), where the miRNA meets its complementary target mRNAs. It is assumed that nucleotides 2–8 of the miRNA, called the seed region, must bind contiguously to a complementary sequence on the target mRNA lying in the 3'-UTR region. Whether miRNA promotes mRNA degradation or translation depends on the degree of complementarity. Perfect or near-perfect complementarity beyond the seed region sequence induces mRNA degradation, whereas imperfect binding results in translational attenuation [43–47]. The multistage maturation process of miRNAs is strictly regulated by many factors, and its deregulation leads to impairment of miRNA expression, giving rise to progression of various diseases [48]. Epigenetic mechanisms are crucial for controlling miRNA expression [49,50]. Several intronic miRNAs are regulated along with their host protein-encoding genes. For example, miR-211 that functions as a melanoma tumor suppressor is an intronic miRNA coexpressed with its host gene, melastatin [51]. Intergenic miRNA expression can also be regulated like protein-encoding genes by DNA methylation or histone modifications [52,53]. Moreover, regulatory circuits may arise through miRNAs that repress the translation of enzymes that are crucial for epigenetic remodeling processes, and this may contribute to the epigenetic control of miRNA synthesis [54]. Some evidence suggests that miRNAs may also exert direct epigenetic functions at the promoter regions through modulating transcription. Mediated by AGO proteins, miRNAs target gene promoters containing complementary

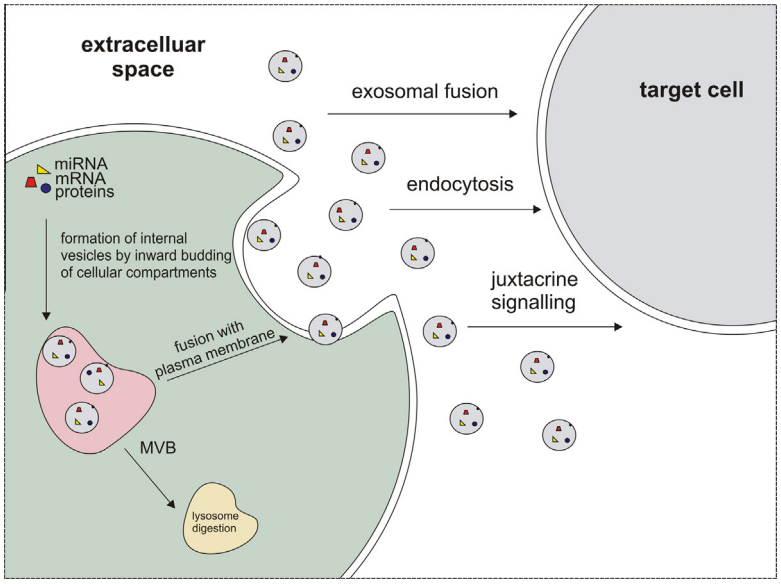

Fig. 1. Exosomal biogenesis and mechanisms of exosomal uptake by target cell. Exosomes, small vesicles formed by inward budding, contain miRNAs, mRNAs and proteins. They are released from the multivesicular body (MVB) when MVB fuses with the plasma membrane. Alternatively MVB can be degraded in lysosomes. Released exosomes can be directly fused to the target cell, releasing its content into the cytoplasm, or they can be endocytized. The third mechanism is the juxtacrine signaling that involves specific receptor proteins that enable the binding of exosomes to the target cells.