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Review

Extracellular RNA mediates and marks cancer progression

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ABSTRACT

Different types of RNAs identified thus far represent a diverse group of macromolecules that are involved in the regulation of different biological processes. RNA is generally thought to be localized primarily in the nucleus and cytoplasm; however, some types of RNA have been detected in the extracellular milieu. These extracellular RNA (exRNA) molecules are protected from degradation and it is now widely accepted that extracellular vesicles and ribonucleoprotein particles serve as transport vehicles for exRNA among cells. The functional consequence of this transfer of genetic information probably encompasses a broad range of normal developmental and physiologic processes in many organisms. This review will focus on the role of exRNA communication in cancer. We will focus on different types of RNA species identified and characterized within tumor-derived extracellular vesicles. Further, we will describe the role of exRNAs in cancer progression, as well as their potential for use as diagnostic biomarkers and therapeutic tools for monitoring and treating cancer, respectively.

1. Introduction

Classical thinking dictates that endogenous RNA is found within the nucleus, where it is transcribed and regulates gene expression, and in the cytoplasm where it participates in protein translation. However, RNA outside of cells – extracellular RNA (exRNA) was identified decades ago suggesting that some RNA molecules are released from cells in a stable form resistant to degradation by ribonucleases (RNAses) [1]. What has become evident over the past few years is that different types of exRNA carried in various types of vehicles are present in the extracellular milieu. More importantly, it has been shown that these exRNA molecules, along with protein cargo, can be transferred between donor and recipient cells and influence the phenotype of the recipient cells [2–6]. This exchange of genetic information between cells with a corresponding change in the phenotype of the recipient cells has been demonstrated in human cancers in several seminal reports over the past few years [3,7–9].

Free-floating RNAs in the extracellular space are highly sensitive to degradation by RNAses found throughout the body; therefore, the ability to detect exRNAs in bodily fluids suggests that they are found in enclosed structures and protected from degradation. Several different vehicles of exRNA transport have been documented [10]. For example, exRNA has been found to associate with high-density lipoprotein (HDL) complexes, the Argonaute 2 complex, and other RNA binding proteins [11–13], as well as extracellular vesicles (EVs) [2,3].

Vesicle release is a naturally occurring process that has been observed in nearly all cell types (for review see [14,15]). EV release occurs not only in most healthy cells, but also in a number of different disease states, including human cancers [2,7,8]. In fact, EVs have been isolated from both cultured cancer cell lines and different biological fluids, including serum, plasma, ascites fluid and urine of cancer patients [3,8,9,16–19]. Most studies have shown that EVs are

Abbreviations: ASO, antisense oligonucleotides; CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; ESCC, esophageal squamous cell cancer; EV, extracellular vesicle; exRNA, extracellular RNA; HDL, high-density lipoprotein; HLSC, human liver stem cell; IDH1, isocitrate dehydrogenase 1; ILVs, intraluminal vesicles; lncRNA, long non-coding RNA; miRNA, microRNA; MSC, mesenchymal stem cells; MVB, multivesicular body; ncRNA, non-coding RNA; piRNA, piwi-interacting RNA; RNAse, ribonuclease; RNP, ribonuclear protein; shRNA, short hairpin RNA; siRNA, short interfering RNA; sncRNA, small non-coding RNA; snoRNA, small nucleolar RNA; SRP, signal recognition particle; Stau1, staufen 1; TE, transposable element; TLR, Toll-like receptor; TNF, tumor-necrosis factor; tRNA, transfer RNA.

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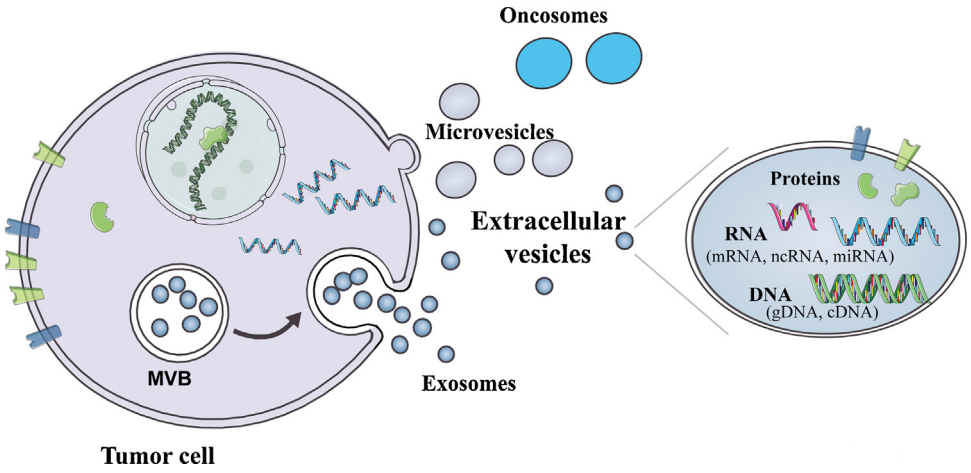

Fig. 1. Biogenesis and cargo of EVs. Extracellular vesicles are comprised of several different types of vesicles including exosomes, microvesicles and oncosomes. Exosomes are formed by the internalization of the endocytic membrane and formation of MVB inside the cell. The fusion of the MVBs with the plasma membrane results in the release of exosomes into the extracellular milieu. Microvesicles are formed by the outward budding of the plasma membrane and are directly released into the extracellular milieu. Oncosomes are a larger type of EV released by cancer cells through budding of the plasma membrane. EVs contain a variety of proteins, RNA species and types of DNA. Modified from [62].

shed in greater numbers from cancer cells, as compared to normal cells [2,20,21].

Extracellular vesicles represent a novel vehicle for cell-cell communication which can allow transfer of cytoplasmic and membrane proteins, as well as DNA and RNA between cells (Fig. 1), and contribute to modulation of numerous biological processes [15,22–27]. Extracellular vesicles can serve as vehicles for transport of exRNA through the extracellular milieu from cancer cells to normal cells in the immediate surroundings as well as distal sites. This novel type of cell-cell communication has emerged as a means for cancer cells to both eliminate RNA and proteins that restrain their growth, and to transfer oncogenic molecules that contribute to progression of cancer and other pathogenic aspects of disease and resistance to therapies [8,9,18,28]. Since the discovery that EVs contain oncogenic molecules that are transferred to recipient cells, research has focused on elucidating the role of EVs in human cancer and the factors within EVs that contribute to disease progression [3,4,7,21,29]. Thus far, cell-to-cell communication mediated by EVs has been shown to be an important contributor in the different stages of cancer progression, such as tissue invasion, immune evasion, angiogenesis and metastasis [9,29–34].

In this review, we will focus on the release of the different types of exRNA molecules including mRNA, microRNA (miRNA), and other non-coding RNA (ncRNAs), as well as transposable elements and how these biomolecules contribute to cancer. Further, we will discuss the effects of exRNAs once they are taken up by recipient cells within the context of human cancers. We will also review the potential of using exRNA as biomarkers and therapeutic vehicles to treat human cancers.

2. Biogenesis and content of extracellular vesicles

Several different types of vesicles have been identified including exosomes, microvesicles, oncosomes and microparticles [7,8,35–38]. The nomenclature within the field of EVs is yet to be precisely defined, thus, in this review we will refer to all types of vesicles as EVs [36]. The different types of EVs identified to date are categorized based on their origin, size and content [37].

Exosomes are the smallest EVs and range from 30 to 100 nm in diameter [39]. They are believed to form by the inward budding of endocytic membranes resulting in the formation of intraluminal vesicles (ILVs), collectively termed multivesicular bodies (MVBs). Exosomes are released into the extracellular milieu upon fusion of the MVB membrane with the plasma membrane (Fig. 1) [35,40]. Microvesicles range in diameter from 100 nm to 1000 nm, are formed by the outward budding of the plasma membrane and are released directly into the extracellular milieu (Fig. 1) [36,37]. Large microvesicles (up to 5 μm in diameter) derived from tumor cells have been termed oncosomes and carry oncogenic molecules that have been shown to alter the phenotype of the recipient cells in support of tumor growth [2,7,8,41].

The content of EVs is diverse and includes proteins, lipids, DNA and different types of RNA [3,21,42,43]. Protein markers have been used to try to distinguish exosomes from microvesicles, but there is some overlap in the content of these two vesicle types. Exosome markers classically include transmembrane proteins CD63, CD81, Alix and Tsg101 [26,37]. Microvesicle protein markers are dictated in part by the proteins on the surface of the cell releasing them and Annexin V is commonly used as a marker [37,44]. The lipid content of the vesicles depends on the type of vesicle being released. For example, the lipid content of exosomes is composed of cholesterol, sphingomyelin and ceramide, while microvesicle membrane has a higher content of cholesterol [35,45].

Several different types of RNA molecules have been detected in EVs including mRNA, long non-coding RNA (lncRNA), small non-coding RNAs (sncRNAs), such as miRNA, and ribosomal RNA [4,46–49]. Some RNA molecules are enriched in EVs, compared to parental cells [e.g. 2,3,50]. RNA messages contained within EVs can be delivered to recipient cells and be translated into functional protein within the donor cell, albeit this may depend on the size and other properties of the RNAs [3,51]. Smaller miRNAs can be efficiently transferred in EVs and frequently appear to be functional in recipient cells [52–54]. The abundance of exRNA within EVs led to the concept of using EV-exRNA as biomarkers for human cancer given that tumor specific RNA, such as EGFRvIII transcript, was identified within EVs [3,48]. We will focus on specific types of RNA species detected within EVs in more detail in sections below. Since most EV preparations are obtained by differential centrifugation with the collection by ultracentrifugation yielding vesicles of all sizes, as well as protein aggregates and HDLs, preparations referred to as EVs may contain exRNA in other forms as well.