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Review

Exosomes as a liquid biopsy for lung cancer

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ABSTRACT

In lung cancer and other malignancies, the so-called "liquid biopsy" is quickly moving into clinical practice. Its full potential has not yet been fully identified, but the "liquid biopsy" is no longer a promise but has become a reality that allows for better treatment selection and monitoring of lung cancer. This emerging field has significant potential to make up for the limitations of the traditional tissue-derived biomaterials. Exosomes are spherical nano-sized vesicles with a diameter of 40–100 nm and a density of 1.13–1.19 g/ml. In both physiological and pathological conditions, exosomes can be released by different cell types, including immune cells, stem cells and tumor cells. These small molecules may serve as promising biomarkers in lung cancer "liquid biopsy" as they can be easily obtained from most body fluids. In addition, the lipid bilayer of exosomes allows for stable cargoes which are relatively hard to degrade. Furthermore, the composition of exosomes reflects that of their parental cells, suggesting that exosomes are potential surrogates of the original cells and, therefore, are useful for understanding cell biology. Previous studies have demonstrated that exosomes play important roles in cell-to-cell communication. Moreover, tumor-derived exosomes are evolved in tumor-specific biological process, including tumor proliferation and progression. Recently, a growing number of studies has focused on exosomal cargo and their use in lung cancer genesis and progression. In addition, their utility as lung cancer diagnostic, prognostic and predictive biomarkers have also been studied. The current review primarily summaries lung cancer-related exosomal biomarkers that have recently been identified and discusses their potential in clinical practice.

1. Introduction

With the rapid progress in understanding lung cancer biology, diagnosis and treatment strategies for advanced lung cancer, in particular non-small cell lung cancer (NSCLC) are currently mainly based on genetic abnormalities. Over the past decades, many correlational and functional biomarkers for lung cancer have been discovered. These include for example, epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and c-ros oncogene 1 (ROS1), which are all functional biomarkers for NSCLC with targetable inhibitors. To date, most of the biomarkers that have been studied in lung cancer, belong to the group of correlational biomarkers that need to be further validated before being applied in the clinic.

In clinical practice, tumor tissue samples are considered the "gold standard" for diagnosis and molecular testing. Unfortunately, there are still major concerns about the results of tissue samples in the era of precision medicine. First, the limited quality and quantity of tissue-derived biomaterials cannot fully meet the criteria for accurate and

Abbreviations: Alix, ALG 2 interacting protein X; ALK, anaplastic lymphoma kinase; AREG, Amphiregulin; Atg2, autophagy related protein 2; AUC, area under the curve; BAL, bronchoalveolar lavage; circRNA, circular RNA; CSE, cigarette smoke extract; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; DDP, cisplatin; DFS, disease-free survival; DR5, death receptor 5; EGFR, epidermal growth factor receptor; EMT, epithelial mesenchymal transition; ESCRT, endosomal sorting complex required for transport; EV, extracellular vesicle; hAD-MSC, human adipose tissue-derived mesenchymal stem cell; HBEC, human bronchial epithelial cells; HSP 70, heat shock protein 70; hTERT, human telomerase reverse transcriptase; HMC-1, human mast cell line 1; lncRNA, long non-coding RNA; LRG1, leucine-rich α-2-glycoprotein; miR/miRNA, microRNA; MSC, mesenchymal stem cells; mTOR, mammalian target of rapamycin; MVB, multivesicular body; NSCLC, non-small cell lung cancer; P-MSC, pro-inflammatory phenotype in mesenchymal stem cells; RANKL, Receptor-Activator-of-Nuclear-factor-Kappa-B-Ligand; ROS1, c-ros oncogene 1; SCF, Stem cell factor; SNARE, Soluble N-ethylmaleimide-Sensitive-Factor Attachment Receptor; STAT3, signal transducers and activators of transcription 3; TEP, tumor-educated platelet; TGF-β, transformation growth factor β; TNM, tumor-nodule-metastasis; TSG101, tumor susceptibility gene 101; VEGF, vascular endothelial growth factor; ZEB1, Zinc finger E-box-binding homeobox-1; ZO-1, Zonula occludens-1

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reliable detection. The small size of the tissue samples may be inadequate for both pathological and molecular testing purposes. Moreover, due to the heterogeneous nature of a tumor, taking one biopsy may fail to provide a complete picture of the entire tumor landscape [1]. Second, repeatedly taking biopsies to acquire tissue samples for initial diagnosing or dynamical monitoring is hard to achieve clinically, especially in patients in which the performance status is poor [2]. These examinations are invasive and may technically be challenging, for example in tumor-containing blood vessels or at locations in the tumor where necrosis occurs. Therefore, novel biomaterials are clearly warranted.

Taking "liquid biopsies" is a new concept that has recently been put forward and involves the collection of body fluids, for example, blood, urine, effusions, or saliva through a minimally invasive method and will undergo clinical testing. This emerging technology has the potential to bypass the above limitations of tumor biopsy. Currently, there are four major strategies for liquid biopsy using blood samples, namely circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), tumor-educated platelet (TEP) and extracellular vesicles (EVs). In addition, cell-free RNA or miRNA may also be potential approaches for using liquid biopsies in lung cancer. EVs are small membranous vesicles that are secreted by either normal or tumor cells. According to the biogenesis and mechanism of secretion, EVs are categorized into the following three subclasses: exosomes, microvesicles, and apoptotic bodies [3,4]. Recently, a cancer-derived EV population termed "large oncosomes" was described. This population was much larger than most EV types characterized to date (1–10 μm diameter). Exosomes play an important role in cancer progression and serve as diagnostic, prognostic, or predictive biomarkers for lung cancer (Fig. 1).

In this review, we will briefly introduce exosomes and their characteristics. We will then focus on the lung cancer-related exosomal biomarkers that have been identified and discuss their potential use in clinical practice. Studies on exosomal cargo were divided into two categories: (I) clinical biomarker studies using clinical samples (human level) and (II) mechanistic studies using preclinical samples (cell and animal level). This review separates the two parts to clearly present the current research knowledge of the two fields. In addition, exosomes that act as a potential drug delivery system in lung cancer treatment are also reviewed.

2. Characteristics of exosomes

Exosomes were first discovered and termed by Johnstone et al. [5] when studying the maturation process of reticulocytes. Exosomes are spherical nano-sized vesicles, and a special class of EVs, with a diameter of 40–100 nm and a density of 1.13–1.19 g/ml [6]. In both physiological and pathological conditions, exosomes can be released by several cell types, including immune cells, stem cells, and tumor cells [7–9].

It is well known that exosomes are generated through the endocytic pathway and are released from the plasma membrane into the extracellular environment via multivesicular bodies (MVBs) that are formed from early endosome maturation under the regulation of an endosomal sorting complex required for transport (ESCRT) and related proteins [10,11]. MVBs can fuse with lysosomal or plasma membranes. The fusion of MVBs with lysosomes leads to the degradation of MVB contents, whereas the fusion of MVBs with the plasma membrane induces the release of MVB contents, including exosomes into the extracellular space [12,13]. The formation of exosomes is linked to cell-secretion and involves several proteins, such as Rab proteins (Rab27A/B), heat shock protein 70 (HSP70), tumor susceptibility gene 101 (TSG101), ALG-2 interacting protein X (Alix), and tetraspanins (CD9, CD63, CD81 and CD82), which are recognized as exosomal markers for identifying true exosomes [11,14]. Due to their endocytic origin, the composition of exosomes reflects that of their parental cells, exosomes represent potential surrogates of the original cells for understanding cell biology. Furthermore, the lipid bilayer of exosomes results in cargo that is stable and relatively hard to degrade, and allows for the identification of the original cells [15].

The function of exosomes is dependent on their parental cytotypes and contents. Normal cell-derived exosomes play a role in maintaining stable homeostasis, whereas tumor cell-derived exosomes are related to tumor progression. Exosomal contents that have been previously identified can be found in online databases, such as ExoCarta (www.exocarta.org) [16], Vesiclepedia (www.microvesicles.org) [17], and EVpedia (student4.postech.ac.kr/evpedia2_xe/xe/index.php?mid=Home) [18]. In general, these nano-sized vesicles contain many molecules including nuclear acids (e.g. double-stranded DNA and various subtypes of RNA), proteins and lipids. Molecules that are packaged into exosomes are highly selective and controlled by ESCRT. Importantly, exosomes released by donor cells can be taken up by receptor cells in an autocrine, paracrine, or exdocrine manner, indicating the key role of exosomes in cell-to-cell communication [19–21]. The transfer of functional content of exosomes to receptor cells can result in physiological or pathological effects [4]. In tumor cells, ESCRT is significantly altered and may cause the molecular profile inside exosomes to be largely modified [11,22]. As a result, exosome-mediated communication within the tumor environment affected tumor-related pathways and contributed to tumor genesis and progress in many types of cancer, including lung cancer [23].

Since exosomes can be easily acquired from most body fluids and characterized, they may serve as a promising "liquid biopsy" biomarkers of lung cancer [24]. Ultracentrifugation-based technologies as well as commercially available kits are methods that are most commonly used for exosome extraction [25].

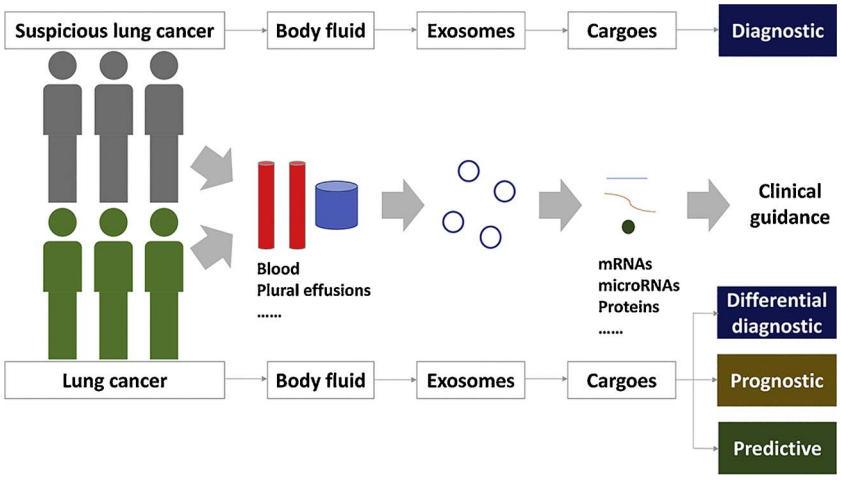

Fig 1. Potential role of exosomes as a liquid biopsy for lung cancer. For suspicious lung cancer individuals, exosomal biomarkers will be identified to facilitate diagnosis (upper panel). For patients who were already diagnosed with lung cancer, relevant exosomal markers will be identified to further monitor and guide their individualized therapies (lower panel).