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Perspective Alternative Cancer Treatments

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Abstract

Extra vesiculars are classified in two categories based on their biogenesis. Specifically, exosomes are small vesicles from endosomes in physiological and pathological conditions and released by a fusion of multi-vesicular bodies to the cell membrane, while shed micro-vesicles, with a typical size present in almost any extracellular bodily fluid and are responsible for the exchange of molecular materials between cells.

Keywords: Molecular materials; Cancer diagnosis; Regimens; RNA; Preclinical practice; Hormonal therapy

Introduction

Exosomes are involved in cancer development and spreading, in the bidirectional communication between tumour cells and surrounding tissues, and in the construction of the micro environment needed for pre-metastatic niche establishment and metastatic progression. Hence, circulating vesicles are clinically relevant in cancer diagnosis, prognosis and follow up. Exosomes are actually recognised as valid diagnostic tools, but they can also be isolated and exploited as anti-cancer vaccines or nano-sized drug carriers in cancer therapy [1]. Nowadays, one of the main issues in cancer diagnosis is the early identification of biomarkers by non-invasive techniques. Obtaining a significant amount of information, before and during tumour treatment, should allow the monitoring of cancer progression and the efficacy of therapeutic regimens. Liquid biopsies to detect circulating tumour cells, RNAs, DNAs and exosomes have been used as indicators for personalised medicine. In recent years, exosomes detection has been validated as a reliable tool for preclinical practice in different cancer types, thanks to the identification of their content, double-stranded DNA, messenger RNA, micro RNA, long non-coding RNA, proteins and lipids. DsDNA has been detected in exosomes isolated from plasma and serum of different cancer cell types, and mutated genes involved in tumour genesis, such as mutated KRAS and TP53, have been identified as disease predictors. Similarly, exosomal AR-V7 mRNA has been used as a prognostic marker of resistance to hormonal therapy in metastatic prostate cancer patients. Gene expression profiling of multiple RNAs from urinary exosomes has been adopted as an efficient diagnostic tool [2]. LncRNAs isolated from serum exosomes have been exploited for disease prognosis in colorectal cancer patients, and multiple miRNAs allow one to distinguish between different lung cancer subtypes. GPC1-positive exosomes have been employed to detect pancreatic cancer, while circulating exosomal macrophage migration inhibitory factor was able to predict liver metastasis onset. Finally, multiple lipids present in urinary exosomes have been approved as prostate cancer indicators. Due to the high variability of patient classes and sample size, and in order to obtain clinically significant results for a fast and effective diagnosis, huge investments in exosome research will be required in the near future.

Discussion

Exosomes could also be exploited as natural, biocompatible and low immunogenic nano-carriers for drug delivery in cancer therapy. They can be passively loaded by mixing purified vesicles with small drugs, or actively loaded by means of laboratory techniques, such as electroporation and sonication [3]. Super paramagnetic nanoparticles conjugated to transferrin have been tested for the isolation of exosomes expressing transferrin receptor from mice blood. After incubation with doxorubicin, they have been used to target liver cancer cells in response to external magnetic fields, inhibiting cell growth both in vitro and in vivo. Kim et al. engineered mouse macrophage-derived exosomes with aminoethyl anisamide-PEG to target sigma receptor, overexpressed in lung cancer cells and passively loaded them with paclitaxel [4]. These systems acted as targeting agents able to suppress metastatic growth in vivo. Three clinical trials with loaded exosomes are currently on-going for the treatment of different tumours : a phase I trial is evaluating the ability of exosomes to deliver curcumin to normal and colon cancer tissues; a phase II trial is investigating the in vivo performance of autologous tumour cell-derived micro particles carrying methotrexate in lung cancer patients and a clinical inquiry is focusing on autologous erythrocyte-derived micro particles loaded with methotrexate for gastric, colorectal and ovarian cancer treatment. Recently, new strategies to produce ad hoc exosomes have been developed. Cells releasing exosomes have been genetically engineered to overexpress specific macromolecules, or modified to release exosomes with particular targeting molecules. Exosomes derived from different cancer cells have already been exploited as cancer vaccines [5]. Autologous dendritic cell-derived exosomes with improved immune-stimulatory function have been tested in a phase II clinical trial for the activation of CD8 + T cells in non-small cell lung cancer patients, observing disease stabilisation and a better overall survival. In a phase I trial, ascites-derived exosomes supplemented with granulocyte-macrophage colony stimulating factor have been administered to colorectal cancer patients, soliciting a tumour-specific immune response. Many issues related to exosomes clinical translation remain open and are mostly connected to the definition of preclinical procedures for isolation, quantification, storage and standard protocols for drug loading. It is becoming even more necessary to distinguish between tumour and healthy blood cell-derived vesicles to characterise their post-isolation half-life and to perform standard content analyses. For these purposes, innovative approaches and technologies have been set up, such as microarrays and specific monoclonal antibodies and RNA markers amplification

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strategies. Natural antioxidants in cancer therapy every day, the human body undergoes several exogenous insults, such as ultraviolet rays, air pollution and tobacco smoke, which result in the production of reactive species, especially oxidants and free radicals, responsible for the onset of many diseases, including cancer. These molecules can also be produced as a consequence of clinical administration of drugs, but they are also naturally created inside our cells and tissues by mitochondria and peroxisomes, and from macrophages metabolism, during normal physiological aerobic processes [6]. Oxidative stress and radical oxygen species are able to damage DNA genetic alterations, DNA double strand breaks and chromosomal aberrations and other bio-macromolecules, such as lipids and proteins significantly changing the regulation of transcription factors and, as a consequence, of essential metabolic pathways. The protective mechanisms our body has developed against these molecules are sometimes insufficient to counteract the huge damages produced. Recently, in addition to research into the roles of the physiological enzymes superoxide dismutase, catalase and glutathione peroxidase, natural antioxidants such as vitamins, polyphenols and plant-derived bioactive compounds are being studied in order to introduce them as preventive agents and potential therapeutic drugs. These molecules have anti-inflammatory and anti-oxidant properties and are found in many vegetables and spices. Vitamins, alkaloids, flavonoids, carotenoids, curcumin, berberine, quercetin and many other compounds have been screened in vitro and tested in vivo, displaying appreciable anti-proliferative and pro-apoptotic properties, and have been introduced as complementary therapies for cancer. Despite the advantages of using natural drugs, their translation into clinical practice remains difficult due to their limited bioavailability and toxicity [7]. Curcumin, a polyphenolic compound extracted from turmeric, is a traditional Southeast Asian remedy with anti-inflammatory, anti-oxidant and chemo-preventive and therapeutic activities. It has been shown to have cytotoxic effects in different kinds of tumours, such as brain, lung, leukaemia, pancreatic and hepatocellular carcinoma, with no adverse effects in normal cells at the effective therapeutic doses. Curcumin can modulate a plethora of cellular mechanisms, however, its biological properties, and as a consequence, the treatment duration and the efficient therapeutic doses, have not been completely elucidated yet. This molecule is highly lipophilic, poorly soluble in water and not very stable [8]. Different strategies and specific carriers, such as liposomes and micelles, have been developed to improve its bioavailability. Currently, few clinical trials involving curcumin are on-going and few have been already completed. Berberine is an alkaloid compound extracted from different plants, such as Berberis. Recently, it has been demonstrated to be effective against different tumours and to act as a chemo-preventive agent, modulating many signalling pathways [9]. Like curcumin, it is poorly soluble in water; therefore, different nano-technological strategies have been developed to facilitate its delivery across cell membranes, six clinical trials are open and one has been completed. Quercetin, a polyphenolic flavonoid found in fruits and vegetable, has been proven to be effective to treat several tumours, such as lung, prostate, liver, colon and breast cancers, by binding cellular receptors and interfering with many signalling pathways. Interestingly, it has been shown to be effective also in combination with chemotherapeutic agents. Presently, seven clinical trials are open and four have been completed. One of the main problems of conventional cancer therapy is the low specificity of chemotherapeutic drugs for cancer cells. In fact, most drugs act both on healthy and diseased tissues, generating severe side effects. Researchers are putting a lot of effort into finding a way to target only the desired site. Nanoparticles have raised great interest for their tendency to accumulate more in tumour tissues due to the enhanced permeability and retention effect. This process, called passive targeting, relies on the small size of nanoparticles and the leaky vasculature and impaired lymphatic drainage of neoplastic tissues [10]. Passive targeting, however, is difficult to control and can induce multidrug resistance. Active targeting, on the other hand, enhances the uptake by tumour cells by targeting specific receptors that are overexpressed on them. Nanoparticles, for example, can be functionalized with ligands that univocally bind particular cells or subcellular sites. Several kinds of ligands can be used, such as small molecules, peptides, proteins, aptamers and antibodies. Folic acid and biotin are small molecules, whose receptors are overexpressed in tumour tissues.

Conclusion

Several nano-carriers have been functionalized with folic acid to target ovarian and endometrial cancers, folic acid-conjugated polyethylene glycol-poly nanoparticles delivering docetaxel increased drug cellular uptake by human cervical carcinoma cells. Small ligands are cheap and can be linked to nanoparticles by simple conjugation chemistry.

Acknowledgement

None

Conflict of Interest

None

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