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Review

Extracellular vesicles as modulators of the cancer microenvironment

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ABSTRACT

The tumour microenvironment is a highly complex and dynamic tissue. It comprises not only neoplastic cells, but also other resident cells within the milieu such as stroma and vascular cells in addition to a variable cellular infiltrate from the periphery. A host of soluble factors such as growth factors, chemokines, eicosanoids soluble metabolites and extracellular matrix components have been extensively documented as factors which modulate this environment. However, in recent years there has also been growing interests in the potential roles of extracellular vesicles (EV) in many of the processes governing the nature of cancerous tissue. In this brief review, we have assembled evidence describing several distinct functions for extracellular vesicles in modulating the microenvironment with examples that include immune evasion, angiogenesis and stromal activation. Whilst there remains a great deal to be learnt about the interplay between vesicles and the cancerous environment, it is becoming clear that vesicle-mediated communication has a major influence on key aspects of cancer growth and progression. We conclude that the design of future therapeutics should acknowledge the existence and roles of extracellular vesicles, and seriously consider strategies for circumventing their effects *in vivo*.

Contents

1. Introduction

Like most cell types, neoplastic cells release small lipid-bounded vesicles into the extracellular space, but they may do so extensively compared to their non-neoplastic counterparts [1]. Genotoxic, hypoxic, metabolic and other forms of cellular stress [2–4] lead to heightened levels of vesicle secretion, together with alterations in vesicle-cargo molecules. In cancer therefore, where such conditions are particularly rife, the vesicle secretion pathway appears to be a major feature.

Cells can release different types of vesicles, which have been difficult to categorise in a definitive manner [5]. There

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are fundamentally two principal vesicle types under discussion. Microvesicles, which are considered large (>200 nm diameter) and dense, and arise from outward budding of the plasma membrane. Traditionally this process may have been related to a mode of purging regions of damaged membrane from the cell in response to sub-lethal complement attack for example, and is considered by many therefore as a form of debris associated with cellular damage [6]. Exosome vesicles are generally smaller (30–150 nm diameter) [7], have a characteristic density of 1.1–1.2 g/ml [8], and are manufactured within multivesicular endosomes of the late endocytic tract [8]. Small (~100 nm) plasma-membrane derived vesicles have also been reported [9]. Categorising vesicles based on their size or subcellular origin therefore remains problematic. Furthermore defining them on the basis of molecular cargo is not straightforward due to the likely overlap between different types of vesicles. Methods such as nano-particle tracking that facilitate the counting of small particulate material invariably demonstrate the predominance of the smaller types of vesicles present in biological fluids or in cell-conditioned media [10]. Whether or not one type of vesicle is biologically more significant than another is simply unclear from our current understanding, hence, the term extracellular vesicles (EV) has been adopted by the field as these questions continue to be investigated.

The transmission of EV from cancer cells to other cell types has been the subject of intensive studies in recent years. It is a process which offers a sophisticated form of cellular communication through the delivery of highly complex and dynamic cargo, packaged within a readily captured vesicle. Recipient cells usually uptake EV through endocytic processes [11], and/or for microvesicles through membrane fusion events [12,13]. Cells receive not only classical receptor–ligand interactions from EV, but do so in the context of co-delivered factors including proteins, lipids and RNA. Hence the biological effects of EV delivery can be profound, as well as difficult to study and characterise. Nevertheless there are many well characterised examples of EV functions in cancer, many of which may indeed become viewed as a coordinated set of mechanisms that act to promote disease.

2. Immunological control by EV

2.1. Immune activating EV

A key discovery by Raposo et al. described the first biological effect of EV interaction with a recipient cell [8]. The study showed the capacity for EV to mimic the function of the parent cell, in this case B-lymphocytes, by stimulating T cell proliferation in an antigen and MHC-restricted manner. Hence the concept of EV-based vaccine therapeutics in cancer was born, and several studies followed demonstrating the potential for EV principally of dendritic cell origin to prime T cell responses against tumour cells [14–18]. In addition, EV from cancer cells harbour tumour-specific antigens, and these pulsed onto dendritic cells could also be a means of eliciting anti-tumour immunity [14,19], in a manner that is advantageous compared to soluble forms of antigen [20]. Modulating tumour cells in various ways, such as heat shock [21] or by forced expression of certain inflammatory factors [22] can render the delivery and cross-presentation of exosomal-antigen more efficient through maturation of the dendritic cells. Components of innate immunity, such as NKT cells may in addition be able to bolster the efficacy of exosome-based vaccines [23]. However, despite such translationally focussed studies which continue to evolve, there is mounting evidence pointing to a predominantly immune-suppressive function for exosomes of cancer cell origin.

2.2. Lymphocyte inhibition by EV

Several diverse mechanisms have been reported by which EV directly participate in tumour immune evasion, with some dramatic effects such as the induction of T cell death. Among the earliest such observations was the description of EV of melanoma cells, which express Fas-ligand (CD95L) on the outer vesicle surface. When encountering Fas-positive (CD95) lymphocytes, these EV induce apoptotic death [24]. This was also confirmed as a phenomenon in colorectal cancer [25] and as a property of vesicles isolated from the sera of ovarian cancer patients [26]. In nasopharyngeal carcinoma, a tumour related to Epstein Barr virus (EBV) infection, circulating EV exhibit high levels of Galectin-9, which mediates interactions with $CD4^+$ helper T cells through the Tim-3 receptor. This is again related to apoptotic death of a subset of T cells that would otherwise participate in tumour, or EBV-specific immune responses [27].

Immune-effector cells do not always undergo death in response to EV. Several examples of functionally important changes in the protein expression profiles of lymphocytes have been reported. The expression of the CD3/T cell receptor complex for example becomes perturbed, in a Fas-ligand related mechanism leading to suboptimal function of surviving T cells [26,28]. The c-type lectin NKG2D receptor, present on $CD8^+$ T cells, NK cells and γδ-T cells is an important mechanism for recognising and responding to virally infected cells, and tumours [29]. However, in addition to proteolytic cleavage of the ligands from the tumour cell surface [30], the ligands are also actively secreted in the form of EV [31] which together with vesicular transforming growth factor beta-1 (TGFβ1), down-regulate NKG2D expression levels, negatively impacting cytokine secretion and cytotoxic functions of $CD8^+$ T cells and NK cells [32]. This particular mode of immune-control is also documented as a foetal protective mechanism during pregnancy [33], and exhibits both local and systemic effects. Other studies also point to impaired NK cell function following exposure to murine breast cancer EV, resulting in defective NK-cell mediated tumour clearance *in vivo* [34], although the molecular basis for this is not fully defined. The NK cell response can also be modulated by EV in haematological malignancies, where NK-activity against CLL was negatively impacted following dysregulated EV-expression of the ligand of NKp30 (termed BAG6/BAT3), with reduced vesicular BAG6/BAT3 leading to immune evasion in a xenograft model [35].

As well as directly impacting effector cells, cancer derived EV can also modulate the regulatory arm of the immune system. This was first demonstrated with pleural malignant mesothelioma derived EV's which strongly inhibited the proliferative response of $CD8^+$ T cells to interleukin-2 (IL-2), partly by activating the suppressive function and elevating the numbers of Foxp-3 positive T^{regs} [36]. This lead to the discovery that TGFβ1 is present at the vesicle surface of certain cancer derived EV, and was responsible for their antigen independent effects on T^{regs} [36], and in some cases interleukin-10 (IL-10) may also be involved [37]. This phenomenon is now acknowledged by several groups [37,38], and has been confirmed with EV isolated from malignant effusions [39]. In fact, such is the importance of vesicular-$TGF\beta_1$ in controlling immune responses that manipulating vesicles to express heightened $TGF\beta_1$ levels may be a novel strategy to control autoimmunity, impacting not only T^{reg} functions but also countering inflammatory Th1 and Th17 T cell responses [40]. EV secreted by mesenchymal stem cells (MSC) thought to contribute to changes within the cancer stroma, may also exhibit inhibitory mechanisms involving TGFβ1, Galectin-1 and PDL-1 present on the EV surface, and hold potential for therapeutic use in autoimmune conditions [41].

EV, however, are also able to exert a more selective suppressive effect, through induction of antigen-specific tolerance. Elegant studies by Robbins et al. may contradict some of the

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