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Review

Extracellular vesicle communication pathways as regulatory targets of oncogenic transformation

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ABSTRACT

Pathogenesis of human cancers bridges intracellular oncogenic driver events and their impact on intercellular communication. Among multiple mediators of this 'pathological connectivity' the role of extracellular vesicles (EVs) and their subsets (exosomes, ectosomes, oncosomes) is of particular interest for several reasons. The release of EVs from cancer cells represents a unique mechanism of regulated expulsion of bioactive molecules, a process that also mediates cell-to-cell transfer of lipids, proteins, and nucleic acids. Biological effects of these processes have been implicated in several aspects of cancer-related pathology, including tumour growth, invasion, angiogenesis, metastasis, immunity and thrombosis. Notably, the emerging evidence suggests that oncogenic mutations may impact several aspects of EV-mediated cell–cell communication including: (i) EV release rate and protein content; (ii) molecular composition of cancer EVs; (iii) the inclusion of oncogenic and mutant macromolecules in the EV cargo; (iv) EV-mediated release of genomic DNA; (v) deregulation of mechanisms responsible for EV biogenesis (vesiculome) and (vi) mechanisms of EV uptake by cancer cells. Intriguingly, EV-mediated intercellular transfer of mutant and oncogenic molecules between subpopulations of cancer cells, their indolent counterparts and stroma may exert profound biological effects that often resemble (but are not tantamount to) oncogenic transformation, including changes in cell growth, clonogenicity and angiogenic phenotype, or cause cell stress and death. However, several biological barriers likely curtail a permanent horizontal transformation of normal cells through EV-mediated mechanisms. The ongoing analysis and targeting of EV-mediated intercellular communication pathways can be viewed as a new therapeutic paradigm in cancer, while the analysis of oncogenic cargo contained in EVs released from cancer cells into biofluids is being developed for clinical use as a biomarker and companion diagnostics. Indeed, studies are underway to further explore the multiple links between molecular causality in cancer and various aspects of cellular vesiculation.

Contents

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1. Introduction – 'pathological connectivity' as a hallmark of cancer

There is a tension between the 'oncogene paradigm' that still organizes our global understanding of cancer causality [1] (mostly from within a cancer cell) and the realisation that virtually all meaningful events underlying malignant disease occur at the multicellular or organismal level. How can an inherited or accidental mutation of a single gene or their panel, in a single cell, impact myriads of surrounding cells, tissues, systemic homeostasis, life and death of an individual?

The likely explanation lies in the realisation that molecular programs of billions of cells populating multicellular organisms are highly integrated, and so are the mechanisms of their derived disease states, including cancer [2]. Indeed, while similarities or convergences may exist between core intracellular signalling pathways involved in progression of human cancers [3,4], their mutational, epigenetic and phenotypic landscapes are extremely different, including the heterogeneous composition of bulk tumour cell populations, cancer stem cell pools [5], stroma [6], vascular niche [7,8], and the associated microenvironmental, inflammatory and immune responses [9,10]. Thus, it could be argued (and documented [9,11–13]) that the web of still poorly defined intercellular communications co-evolves with, and enables the biological manifestations of oncogenic mutations during progression of individual malignancies. Furthermore, scarcely understood pathological 'quorum' responses, 'field effects', and 'cellular ecosystems' [14] likely represent a distinct form of disease causation and a virtually unexplored therapeutic target.

Context-dependent responses, cellular interaction, competition and cooperation between cellular populations in cancer are neither novel nor esoteric [14]. The collective rather than single-cell based determination of biological events in cancer is exemplified by studies on cellular interactions during tumour onset [15], cancer cell growth and survival [16,17], cellular composition and heterogeneity [13], metastasis [18–20], drug resistance [21,22], angiogenesis [7,23–25] and many other central aspects of malignant progression [26].

While the existence of such interactive processes is increasingly well documented [27–29], their extensive and systematic mapping still needs to be more fully explored. The relative torpidity of these efforts stands in contrast to massive molecular profiling programs currently underway in relation to virtually all cancer sites and largely predicated on hopes of revealing an ever larger spectrum of putative oncogenic targets for anticancer therapy focusing mainly on the inner workings of a 'cancer cell' [30,31]. Notably (perhaps paradoxically), biochemically active drugs directed at cell-autonomous effects of oncogenic drivers have shown variable therapeutic activities *in vivo* [32,33] and have not, for the most part, been curative in the clinic [34]. In other words, in spite of their potent transforming potential *in vitro,* the genetic and epigenetic driver events present in cancer cells may not fully explain all aspects of the malignant process *in vivo*, or provide sufficient targeting opportunities in the course of anticancer therapy.

Although the incessant genetic drift, instability, clonal selection and drug delivery considerations may account for some of the therapeutic challenges in cancer, it is also possible that the way oncogenic pathways operate in isolated cells versus their interactive cellular communities *in vivo* may be considerably different. For example, while glioblastoma (GBM) cells often exhibit oncogenic activation of the epidermal growth factor receptor (EGFR), the respective EGFR inhibitors have shown rather disappointing results in the clinic [33]. In this regard, recent studies reveal that patterns of EGFR pathway activation and inhibition are different between *in vitro* and *in vivo* conditions, and are markedly influenced by the complexities of the tumour microenvironment they apparently elicit [35].

Indeed, several aspects and mediators of the tumour microenvironment can be traced (but not reduced) to the influence of oncogenic mutations on the cancer cell secretome, as exemplified by molecular regulators of vascular, inflammatory, immune, coagulant, and desmoplastic responses [11,12,36–38]. Conversely, there is ample evidence for reciprocal effects and interactions within tumour and stromal cellular populations [9,23,39]. Among many effectors of this 'pathological connectivity' the role of extracellular vesicles (EVs) stands out, and will be discussed, as a biologically unique, poorly understood and important emerging influence [40].

2. Extracellular vesicles as mediators of intercellular communication in cancer

EVs are heterogeneous structures released from cells as membrane encapsulated slivers of cellular content. They can be shed into the *peri*-cellular space either constitutively or following cell stimulation, stress, transformation or death, processes that they both reflect and influence. The underlying biogenetic mechanisms, molecular profiles, structures, properties and diverse biological roles of EVs in various contexts have been extensively reviewed [40–42], and require but a minimal mention.

Thus, EVs are thought to play unique roles in both cell-autonomous and non-cell-autonomous regulatory processes. The former include extracellular export and depletion ('dumping') of certain components of the cellular content, including macromolecules that are superfluous, or interfere with cellular functions, or are removed because of their involvement in the process of EV formation (vesiculation) *per se* [43–45]. This removal may change the properties of EV-emitting cells [46], including perturbations in the levels of specific microRNA, tumour suppressors or regulatory signals [47–49].

Once exported, EVs come into contact with cells, extracellular matrix (ECM) and surrounding biofluids (e.g. blood) the properties of which they may influence [49–54]. The nature of EV contacts with encountered (recipient) cells may range from external interactions of EVs with cellular surfaces, to membrane fusion and different forms of EV internalization or uptake [55]. Recipient cells may either degrade EVs and their cargo [56], re-emit them in a modified form [57], or respond to their bioactive constituents by biological and functional changes [52,58].

Biological effects of EVs are pre-programmed by the identity of their parental cells and processes of EV biogenesis (as well as the properties of recipient cells). Indeed, while EVs may to some extent, reflect the molecular make-up of their parental cells, their content is also a function of still poorly understood selective molecular 'packaging' mechanisms [59–62]. Thereby EVs assume the role of unique portals for extracellular release and intercellular transfer of secretable and non-secretable biological regulators, intracellular and integral membrane proteins, as well as cellular mRNA, microRNA, other non-coding RNA species, and DNA [41,42,63].

The EV-mediated intercellular transfer of molecules (communication) occurs both locally and systemically, and possesses several distinctive features. Packaging of cellular macromolecules into EVs protects this cargo from degradation and maintains its integrity, composition and activity in the circulation, while directing it to defined cellular recipients capable of EV uptake [27,54,64,65]. In keeping with this notion, recent studies show that even minimal amounts of EV-associated transcripts encoding potent indicator enzymes, such as *Cre* recombinase or *Gaussia* luciferase may provoke detectable responses in EV recipient cells, even at distant anatomical locations [54,64,66,67]. The significance of systemic trafficking of exosomes in cancer has been recently reinforced by