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Review

Extracellular vesicles in gastrointestinal cancer in conjunction with microbiota: On the border of Kingdoms

Natasha S. Barteneva[a,*], Yeldar Baiken[b], Elizaveta Fasler-Kan[c,d], Kenneth Alibek[b,1], Sheng Wang[e,2], Natalia Maltsev[e], Eugene D. Ponomarev[f], Zarina Sautbayeva[b], Sholpan Kauanova[b], Anna Moore[g], Christoph Beglinger[h,i], Ivan A. Vorobjev[b]

[a] Department of Pediatrics, Harvard Medical School and Boston Children Hospital, Boston, USA
[b] School of Science and Technology, Nazarbayev University, Astana, Kazakhstan
[c] Department of Biomedicine, University of Basel and University Hospital Basel, Basel, Switzerland
[d] Department of Pediatric Surgery, Children's Hospital, Inselspital and Department of Clinical Research, University of Bern, Bern, Switzerland
[e] Department of Human Genetics and USA Computation Institute, University of Chicago, Chicago, USA
[f] School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong
[g] Molecular Imaging Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, USA
[h] Department of Biomedicine, University of Basel, University Hospital Basel, Basel, Switzerland
[i] Division of Gastroenterology, University Hospital Basel, Basel, Switzerland

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ABSTRACT

Extracellular vesicle (EV) production is a universal feature of metazoan cells as well as prokaryotes (bMVs - bacterial microvesicles). They are small vesicles with phospholipid membrane carrying proteins, DNA and different classes of RNAs and are heavily involved in intercellular communication acting as vectors of information to target cells. For the last decade, the interest in EV research has exponentially increased though thorough studies of their roles in various pathologies that was not previously possible due to technical limitations. This review focuses on research evaluating the role of EV production in gastrointestinal (GI) cancer development in conjunction with GI microbiota and inflammatory diseases. We also discuss recent studies on the promising role of EVs and their content as biomarkers for early diagnosis of GI cancers.

The bMVs have also been implicated in the pathogenesis of GI chronic inflammatory diseases, however, possible role of bMVs in tumorigenesis remains underestimated. We propose that EVs from eukaryotic cells as well as from different microbial, fungi, parasitic species and edible plants in GI tract act as mediators of intracellular and inter-species communication, particularly facilitating tumor cell survival and multi-drug resistance.

In conclusion, we suggest that matching sequences from EV proteomes (available from public databases) with known protein sequences of microbiome gut bacteria will be useful in identification of antigen mimicry between evolutionary conservative protein sequences. Using this approach we identified *Bacteroides* spp. pseudokinase with activation loop and homology to PDGFRα, providing a proof-of-concept strategy. We speculate that existence of microbial pseudokinase that 'mimics' PDGFRα may be related to PDGFRα and *Bacteroides* spp. roles in colorectal carcinogenesis that require further investigation.

Abbreviations: AchE, acetylcholinesterase esterase; Ago, Argonaute protein family, essential catalytic components of RISC; AnV, annexin V; APC, antigen-presenting cells; ARF6, ADP-ribosylation factor 6; bft, *Bacteroides fragilis* toxin; bMVs, bacterial microvesicles; BPD, benign pancreatic disease; CEA, carcinoembryonic antigen; CEC, colonic epithelial cells; CrD, Crohn's disease; CRC, colorectal cancer; DAPI, (4′,6-diamidino-2-phenylindole); DHR, dihydrorhodamine; DMEM, Dulbecco's modified Eagle medium; ECM, extracellular matrix; EGFP, enhanced green fluorescent protein; EpCAM, epithelial cell adhesion molecule; EVs, extracellular vesicles; FFPE, formalin-fixed paraffin embedded; FP, fluorescent protein; GI, gastrointestinal; GM1, monosialotetrahexosylganglioside; GPC1, membrane-anchored proteoglycan molecule glypican-1; IBD, inflammatory bowel disease; IEM, immunoelectron microscopy; IFC, imaging flow cytometry; IL, interleukin; KRAS, GTPase, that in human is encodes by the *KRAS* gene; lnsRNAs, long non-coding RNAs; LSPR, localized surface plasmon resonance; MEK-ERK, mitogen-activated protein kinase kinase/extrac membrane-anchored proteoglycan molecule glypican-1ellular-signal regulated kinase; MPs, microparticles; MUC1, mucin1; MVs, microvesicles; MVBs, multivesicular bodies; OD, optical density; OMPs, outer membrane vesicles; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PCA, capsular polysaccharide A; PCD, programmed cell death; PDAC, pancreatic ductal adenocarcinoma; PDGFR, platelet-derived growth factor receptor alpha; PE, phycoerythrin; PI, propidium iodide; PODO, podoplanin; qRT-PCR, quantitative RT-PCR; RFP, red fluorescent protein; RISC, RNA-induced silencing complex; SCID, severe combined immunodeficiency (non-human); SELN, exosome-like synthesized nanoparticles; SMLM, single-molecule localization microscopy; TEV, tumor-originating extracellular vesicles; TNF-alpha, tumor necrotic factor alpha; TF, tissue factor; YSPAN8, tetraspanin 8; VEGF, vascular endothelial growth factor; VTEs, venous thromboembolic events

* Corresponding author at: 200 Longwood Ave, D-249, Boston, MA 02115, USA.
E-mail address: Natasha.Barteneva@childrens.harvard.edu (N.S. Barteneva).
[1] Present address at Locus Solutions Inc., OH 44139, USA.
[2] Present address at King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia.

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1. Introduction

Chronic inflammation pathologies of GI such as inflammatory bowel disease (IBD), Crohn's disease (CrD), *Helicobacter pylori*-associated inflammation and chronic pancreatitis have been identified as strong risk factors for cancer development [1–6]. The initial hypothesis that the emergence of the tumor is associated with chronic inflammation was postulated by Rudolf Virchow in 1863 [7]. Today, the causative link between cancer and chronic inflammation is widely accepted, though molecular and cellular mechanisms of this association have not been resolved [8]. About 15% of the global cancer burden can be attributed to infectious agents [9], of which chronic inflammation is a major component [1]. Acute inflammation is self-limiting, since the production of anti-inflammatory cytokines (IL-1, IL-10, IL-13 etc.) follows the production of pro-inflammatory cytokines (IL-1, TNF-alpha, IFNγ etc.) [8]. The strongest association of chronic inflammation and underlying infection with cancerogenesis is found between inflammatory bowel diseases and colon cancer, *Helicobacter pylori* and gastric cancer, hepatitis C and liver carcinoma, schistosomiasis and bladder and colon carcinomas [10]. The broader implication of these observations was that chemokines and other cytokines are widely involved in cancer development, however, the detailed mechanisms linked to infection and inflammation in relation to cancer are not well understood.

It is well known that cell activation and pathogenesis of a variety of diseases are often associated with increased levels of EVs released in body fluids including plasma, liquor, urine, bile, saliva, semen, vitreous and synovial fluids, atherosclerotic plagues, mucus and intestinal fluids, ascitic and pleural fluids [11–14]. EVs production by eukaryotic cells is upregulated during cell activation and growth, thus playing an essential role in cellular communication during cancer development. However, until recently it was not clear how commensal and pathogenic bacteria, and members of gut microbiota, communicate with other microbial and eukaryotic cells and the immune system. Studies during the last decade that are focusing on the association between gastrointestinal cancer and inflammatory diseases with certain types of bacterial infections often are overlooking the intensive production of outer membrane vesicles (OMPs) by different types of gastrointestinal bacterial commensals and pathogens as well as fungi, parasites invading the GI tract, and nematodes.

This review focuses on research assessing EVs originating from different types of eukaryotic and prokaryotic cells inside the GI tract which separates symbionts and commensals in our bodies. We also discuss the role of EVs originated from GI cancers as potential biomarker tools. Early diagnosis of GI cancers continues to be a major challenge with a miss rate of up to 6.7% - for the upper GI tract with endoscopy and colonoscopy [15] and up to 6% miss rate for colorectal cancers [16]. Due to the invasiveness of these procedures and the possibility of complications [17], there is a high need for robust circulating biomarkers of GI cancers and effective non-invasive monitoring. In conclusion, we suggest that matching sequences from EV proteomes (available from public databases) with known protein sequences of microbiome gut bacteria will be useful in identification new molecular mimicry target protein sequences and provide a proof-of-concept strategy identifying *B. fragilis* pseudokinase that 'mimic' PDGFRα.

2. Extracellular vesicles (EVs) structure and biogenesis

Generally, three different classes of EVs (Fig. 1) are produced by metazoan cells, namely, exosomes, microparticles (MPs) or microvesicles, sometimes also named ectosomes, and apoptotic bodies. These EVs are distinguished by size, their content, morphology and by different mechanisms of their biogenesis [18–19]. Moreover, it is well accepted that production of EVs represents a universal feature of life, since gram-negative and gram-positive bacteria as well as *Archaea* generate outer membrane vesicles [20–24]. It is believed that the formation of microparticles (MPs) usually happens via plasma membrane budding and shedding associated with membrane sites enriched with lipid rafts [25–26,18]. Unlike MPs, exosome formation occurs via rerouting of multivesicular bodies (MVBs) to the cell surface [27–28], where they fuse with the cell membrane and exit the cell through exocytosis (detailed rev. [29]). As a result, exosomes are enriched with endosome- and MVBs-associated proteins, such as tetraspanins (CD9, CD63, CD81 and CD82 and CD151; whereas CD37, CD53 and Tssc6 are restricted by hematopoietic cells) [30–31]. CD63 is important for exosomal secretion, as the reduction of exosomal production was observed in CD63-knockout HEK-293 cells. However, at the same time no reduction in the secretion of microparticles > 150 nm size was noted, supporting the observation that CD63 is important for exosomal

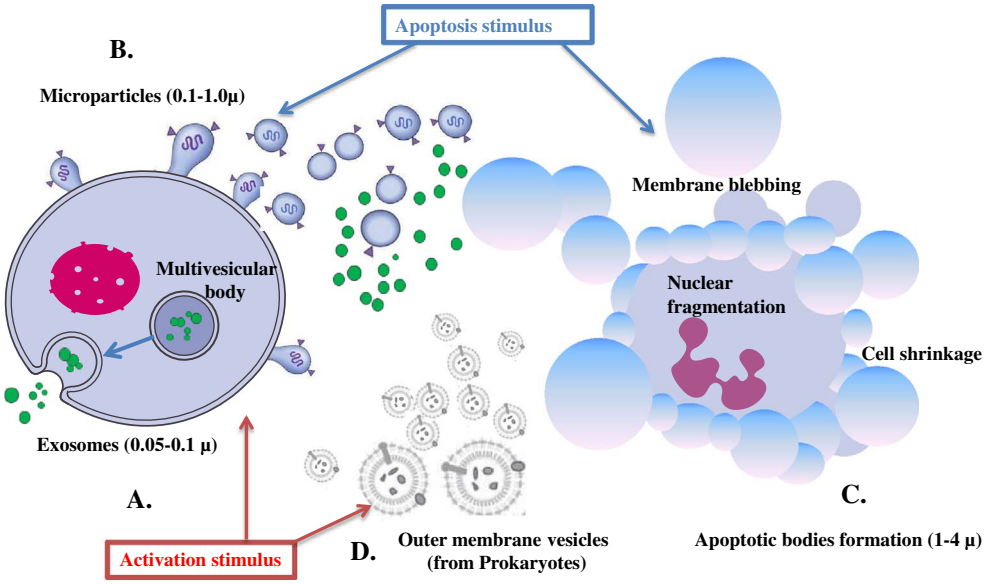

Fig. 1. Production of different classes of EVs. (A) Microparticles; (B) exosomes; (C) apoptotic bodies; (D) outer membrane vesicles.