Editorial

Tumor exosomes: a novel biomarker?

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J Gastrointest Oncol 2011; 2: 203-205. DOI: 10.3978/j.issn.2078-6891.2011.041

Colorectal cancer is diagnosed in approximately one million people worldwide each year, making it the third most common malignancy (1). Not unexpectedly, early diagnosis is associated with a better prognosis. Five-year survival rates of stage I and II colorectal cancer are in excess of 70%. However, in spite of advances in treatment, metastatic colorectal cancer has a five-year survival rate of less than 10% and is not considered a curative disease (2). Among the "holy grails" of cancer diagnostics is the discovery of novel, cost-effective biomarkers of sufficient sensitivity and specificity to permit detection in a more timely fashion. Additionally, biomarkers that provide prognostic information and help clinicians to tailor cancer treatment and monitor response to treatment would be of considerable value. For example, mutations of the *K-Ras* gene, which are present in 40% of patients with metastatic colorectal cancer, have been found to be predictive of a poor response to EGFR-targeted drugs (3). Cellular vesicles are among the newer biomarkers that have been described in the literature, not only for cancer, but for a variety of human diseases.

Cellular vesicles are shed from a variety of cell types and since they contain cell membrane and cytoplasm, their contents are reflections of their cell of origin. Vesicles arising from platelets and red blood cells were among the first to be described several decades ago, but their biological significance was not known initially. Chargaff and West were among the first to report the procoagulant properties of these entities as they observed that the high speed centrifugate of human cell and platelet-free plasma normalized clotting of blood from a patient with hemophilia (4). Since the early descriptions, many different subpopulations of cellular vesicles have been described, including exosomes (5), microvesicles (6), ectosomes (7), membrane particles (8), exosome-like particles (9) and apoptotic vesicles (10). Subpopulations can be distinguished from one another based on size, density, morphology by electron microscopy, sedimentation by ultracentrifugation, lipid composition, protein markers and mechanisms by which they are formed. Exosomes, the subpopulation of vesicles described in this edition of the *Journal of Gastrointestinal Oncology* by Koga et al, are 30-100 nanometers in diameter and are derived from endocytic vesicles. They are released upon fusion of multivesicular bodies with plasma membranes (11). Although there is likely to be some overlap of surface proteins present in different vesicle subpopulations, the tetraspanins, which include CD9, CD81, CD 82 and CD63, are typical components of exosomes (11). Shedding of vesicles occurs in steady state but is increased under the influence of a variety of exogenous stressors including hypoxia, shear stress, irradiation, chemotherapeutic agents and cytokines (12). Conveniently, cellular vesicles can be detected in the circulation and are found in elevated levels in a variety of human diseases including cardiovascular disease, infections, hypertension, diabetes mellitus, Crohn's disease and cancer (13). Cellular vesicle subpopulations are now known to contain DNA (genomic and mitochondrial), mRNA, microRNA and membrane and secreted proteins, some of which help to identify which cell population(s) these vesicles originated from (14). As a result, many have begun to investigate the use of cellular vesicles as disease-specific biomarkers. Taylor et al (15) recently reported that more circulating exosomes could be isolated from patients with ovarian cancer compared with patients with benign

No potential conflict of interest.

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Submitted Sep 01, 2011. Accepted for publication Sep 01, 2011.
Available at www.thejgo.org

ISSN: 2078-6891

ovarian disease and that higher levels were associated with more advanced disease. Several distinct microRNA species could be isolated from these exosomes, eight of which were also found in *ex vivo* tumor samples from the same patients. Importantly, the microRNA profile of these exosomes was different from those isolated from patients without ovarian cancer, suggesting that this profile could act as a "molecular fingerprint" capable of providing non-invasive diagnostic and prognostic information.

A natural extension of studies as such would be to examine patients with known colorectal cancer or those at high risk of developing colorectal cancer as up and down regulation of various microRNA species have been noted in colorectal cancer tissue samples compared with normal colonic tissue (16). In this edition of the *Journal of Gastrointestinal Oncology*, Koga et al point out that one of the technical limitations of RNA-based assays is that RNases are fairly ubiquitous and can rapidly degrade RNA in clinical samples. In this study, the authors examine the durability of exosomes-based microRNA in the face of RNase digestion. Homogenates of feces from healthy volunteers and cultured HT-29 cells (human colorectal cancer cell line) were treated with RNase. Total RNA was extracted from RNase-treated cells (cultured HT-29 or colonic epithelial cells isolated from feces) and exosomes isolated from cell-free HT-29 culture media or feces. Additionally, free RNA from both conditions was isolated. Samples were then analyzed for the presence of selected microRNA species by real-time RT-PCR. Investigators found that free microRNA was completely degraded by the addition of RNase whereas cellular microRNA was resistant to RNase degradation. Interestingly, exosomal microRNAs were partially (HT-29 cell-derived) or completely (feces-derived) resistant to RNase degradation. Among the microRNA species analyzed in this study was miR-21, which has elevated levels in colorectal cancer tissue compared with normal colonic tissue; however, no differences have been noted with respect to early versus advanced stage colorectal cancer (17). Nonetheless, if validated in larger, appropriately-powered studies, findings as such could pave the way to the development of highly sensitive and specific and potentially cost-effective colorectal cancer screening tests, particularly in regions of the world with relatively scarce endoscopic resources.

In this context, exosomes may represent a biomarker of cellular injury or atypia. However, others have demonstrated that these and other cellular vesicles may provide important insights in the pathogenesis of certain diseases, including cancer. Recent interest has focused on their capacity to shuttle cellular components from one cell to another and alter cellular fate. Transfer of membrane receptors between cells has been reported as has transfer of HIV and prions (18-22). Our group has demonstrated that murine lung tissue-derived microvesicles induce co-cultured bone marrow cells to express pulmonary epithelial cell-specific mRNA and protein, likely through the transfer of a microRNA or protein-based transcription factor contained within microvesicles (14,23). When transplanted into lethally-irradiated mice, microvesicle-modified marrow cells preferentially engraft the lung as functioning type II pneumocytes (unpublished findings). *In vitro* culture studies done by our group and others have demonstrated that tumor-derived microvesicles can transfer determinants to non-malignant cells (18) and that human prostate cancer tissue is capable of inducing tissue specific mRNA transcription in human bone marrow cells (24,25). In a similar vein, Al-Nedawia et al. reported that microvesicles produced from human cancer cell lines can transfer EGFR to human umbilical vein endothelial cells, *in vitro* (26). Cancer cell line xenografts in SCID mice that were treated to block microvesicle production had reduced tumor angiogenesis and growth, suggesting a role of tumor-derived microvesicles in cancer progression.

Our understanding of cellular vesicles has grown substantially as evidenced by the number of published reports, which have seemingly grown at an exponential rate over the past decade. Once believed to be cellular cast offs, these intriguing entities are now being viewed as potentially important disease-specific biomarkers, contributors to tissue repair processes and mediators of disease pathogenesis. Their contents are not random but rather provide essential insights of the health status of the originator cell and, perhaps, clues if other cells will be impacted in a beneficial or detrimental fashion.

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