The American Journal of Pathology, Vol. 182, No. 5, May 2013

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

See related article on page 1552.

COMMENTARY

Revealing the Mechanism of Tissue Damage Due to Tobacco Use

Finally, a Smoking Gun?

Philip Furmanski

From the Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy; the Department of Cell Biology and Neuroscience, School of Arts and Sciences; and the Department of Biochemistry and Molecular Biology and Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey; Rutgers University, Piscataway, New Jersey

CME Accreditation Statement: This activity ("ASIP 2013 AJP CME Program in Pathogenesis") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

The ASCP designates this journal-based CME activity ("ASIP 2013 AJP CME Program in Pathogenesis") for a maximum of 48 AMA PRA Category 1 Credit(s)™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

CME Disclosures: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

We have known for more than half a century that tobacco use causes cancers, emphysema, atherosclerosis, and a number of other debilitating and deadly diseases. Some gains have been made in the United States and other countries in reducing tobacco use but it remains a serious worldwide health problem with millions of new cases of tobacco-related diseases diagnosed each year. Remarkably, despite its importance and much study, we still do not have a full picture of the molecular mechanisms by which tobacco, its constituents, and pyrolytic products cause the tissue damage that underlies the well-recognized tobacco-associated pathologies.

In this issue of *The American Journal of Pathology*, Li et al[1] provide a compelling case for a mechanism by which tobacco smoke extract (TSE) induces damage to the extracellular matrix, a key element in the pathogenesis of tobacco-related disease. They find that exposure of human macrophages to TSE initiates a progressive series of events that culminate in apoptosis and the release of small membrane-bound vesicles that carry on their surface a potent collagenolytic and gelatinolytic activity which then degrades the local matrix. Significantly, among the large number of possible proteases that might be involved, they find that only one, matrix metalloproteinase-14 (MMP-14), is responsible for practically all of the activity. Li et al[1] also identify the intracellular signaling pathway that initiates the process after TSE exposure, involving Jun N-terminal kinase (JNK) and p38 MAP kinases.

This study offers important insights and implications regarding the mechanism of tobacco-induced tissue damage and also focuses attention on just a couple of central players in what is likely a complex pathogenetic process: a single transmembrane protease from the MMP family; and a packaging, intercellular communication, and cargo delivery system, the microvesicle (MV), that has gained in visibility, importance, and notoriety over the past few years. So, what is the nature of these TSE-induced MVs, and why do they seem to display only MMP-14? Also, what does all this imply for our understanding and management of tobacco-related disease?

From Cell Debris to Sophisticated Intercellular Communication and Cargo Delivery System

Small, membrane-bound, extracellular vesicles have long been observed in biological fluids and conditioned media. Until

Accepted for publication February 14, 2013.

Address correspondence to Philip Furmanski, Ph.D., Susan Lehman Cullman Laboratory for Cancer Research, Ernest Mario School of Pharmacy, Rutgers University, 164 Frelinghuysen Rd., Piscataway, NJ 08854. E-mail: furmansk@rci.rutgers.edu

http://dx.doi.org/10.1016/j.ajpath.2013.02.004

relatively recently, most observers have considered them uninteresting debris, the dull refuse of cell death, or simply artifact. It is now apparent, however, that vesicles, or at least some of them, are key features of a ubiquitous and highly organized system of cell communication and intercellular material transport.[2–5] Vesicles move cargo from cell to cell that can include enzymes, informational (mRNAs) and regulatory molecules (miRNAs and transcription factors), antigens, membrane receptors, metabolites, and other small molecules. When examined carefully, virtually all cells, including eukaryotic and prokaryotic cells, can be seen to release vesicles. The numbers released constitutively are usually small and are greatly increased by stimulation, activation, or stress. There seem to be several classes of vesicular structures that differ in size and origin. They have been observed and rediscovered many times in association with a broad array of normal physiological processes, and frequently, as is the case with mediators of powerful activities, if produced in a disorderly or uncontrolled fashion, tissue destruction or disease can result. As discussed below, they can even be usurped by malicious cells to advance their own devices.

As often occurs when a cellular component or structure emerges again and again in association with a broad array of positive and negative processes, they initially acquire a wide assortment of names and attributed properties, defying classification and muddying the relationships among them. This certainly has been the case for these small cell vesicles. However, a clarifying order is being brought to what became a menagerie of little particles, helping to advance our understanding of their true functions, origins, and applications.

We now recognize three general categories of these vesicles: i) the ectosomes, ii) the exosomes, and iii) apoptotic bodies.[6] The ectosomes have been variously called microparticles, cell vesicles, nanovesicles, argosomes, prostasomes, prominosomes, apoptotic blebs, and microvesicles, among others. They characteristically arise by budding from the plasma membrane through the process of exocytosis, and generally are 100 to 1000 nm in diameter. Membrane asymmetry is lost during their formation, exposing phosphatidylserine from the inner membrane leaflet on their surface, meaning that they will tightly bind annexin V. The exosomes have also been given many names, including shedding microvesicles, dexosomes, and exovesicles, among others. They arise in the late endosomal system, where they are assembled into multivesicular bodies, which are carried to the cell surface to fuse with the plasma membrane and release their exosomes into the extracellular space. They are typically 40 to 100 nm in diameter, and because of their origin in the endosome compartment they have a different membrane composition from ectosomes, including very low exposure of phosphatidylserine on their surface. The third category, the apoptotic bodies are generally quite large (>1000 nm diameter) and are formed during the late stages of programmed cell death, and also contain many different cellular components, which can include organelles and nuclear fragments, indiscriminately taken up during the dismantling of the dying cell.

Ectosomes contain an array of molecules collected from the cytoplasm and proteins picked up from the plasma membrane that are transferred horizontally to other cells, or, as highlighted here, that are involved in surface or contact-mediated effects on other cells or the cell matrix. Exosomes contain mainly proteins destined for export. Apoptotic bodies are mainly involved in packaging the detritus of cell destruction for uptake by scavenger cells and recycling or elimination.

Vesicles have been assigned to these categories on the basis of size, vesicle content, or association with a physiological stimulus or process (eg, apoptosis). Surface properties, however, such as binding of annexin V, appear to be better markers.[6]

Which vesicle is implicated in mediating the TSE effects? Li et al[1] refer to their vesicles as MVs and also as apoptotic microvesicles or blebs (presumably to be distinguished from apoptotic bodies, which as previously noted are very different structures). The sizes they report for their MVs are consistent with an ectosomal identity, and visualization of their budding from the cell membrane distinguishes them from exosomes. Li et al[1] show that release of the MVs after TSE exposure, but not induction of MMP-14 expression, is associated with the initiation of apoptosis, and is caspase and mitogen-activated protein kinase-dependent. This would place their MVs squarely in the ectosome class, although the authors note that they exhibited only a partial loss of membrane asymmetry, a defining characteristic of ectosomes. Given the indistinct identification and classification of vesicles in many prior studies, further characterization of the TSE-induced MVs using surface markers is warranted, but for now we can accept that they are probably true ectosome-derived MVs.

More on Ectosome MVs

Only small numbers of ectosome MVs (hereafter referred to simply as MVs) are found in the circulation of healthy individuals and their half-life is quite short.[7] The numbers of MVs and their persistence increases greatly with thrombosis, inflammation, sepsis, metabolic disorders, autoimmune diseases, trauma, as well as malaria, sickle cell disease, cancer, and others.[3] They alter endothelial cell function, promote angiogenesis, participate in antigen presentation, and facilitate the development of an immune response.[8–10] MVs deliver MMPs (including MMP-2 and MMP-9), as well as vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived growth factor to sites of angiogenesis.[2,9] They also have a treacherous side and a great deal of attention has been focused in this regard on MVs released by cancer cells, sometimes called oncosomes, which appear to play a role in tumor progression and resistance to therapy. For example, ovarian cancer cells release MVs expressing the Fas ligand, which causes T-cell apoptosis, and could contribute to the escape of tumor cells from immunosurveillance or immunodestruction.[4,11] MVs released from platelets can transfer CD41 to tumor cells, enhancing their