Invited Commentary

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EXOSOMES: CAN DOCTORS STILL IGNORE THEIR EXISTENCE?

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SUMMARY

With this invited commentary we want to draw the attention of young medical doctors, the main readers of this journal, towards the existence and importance of a group of nanovesicles released by human cells: the exosomes. These vesicles are incontinently secreted as a mean of cell-to-cell communication. They are involved in a number of physiologic processes as well as in the pathogenesis of, virtually, all human diseases. They can be isolated from all biological fluids, like blood, urine, sweat, sperm, crevicular fluid, bile, etc., and their composition in terms of proteins, RNA and lipids is different in pathology that in physiologic conditions. It is therefore possible to predict that they will become an important diagnostic and therapeutic tool in medicine.

Cell-to-cell communication is imperative for life. Intercellular communications are mediated through "sending" and "receiving" information via the secretion and subsequent receptor-mediated detection of biomolecular species (1). Each type of cell exhibits a particular set of receptors that allows it to respond to a corresponding number of signal molecules produced by other cells and these ligands act in combination to influence cell behavior (1). There are many pathways of intercellular communication such as the presence of signaling molecules on plasma membranes or secretion of soluble ligands. A number of evidences have now demonstrated new mechanisms of intercellular interaction through lipid vesicles (2, 3).

The extracellular vesicles are constituted by a lipid bilayer that contains in its lumen both transmembrane and free proteins, RNA and microRNA; based on their composition and size these vesicles can be categorized into various classes such as exosomes, apoptotic bodies, microparticles, etc. (4). In recent years many studies have focused on exosomes, a particularly important class of extracellular vesicles (5). Exosomes are 30-100 nm wide membranous vesicles first described in 1984 by Pan and Johnstone in maturing mammalian reticulocytes as a mechanism for removal of plasma membrane proteins (6); over the years they have been observed to be secreted into the culture medium from a large number of cell types, including T cells, B cells, dendritic cells, platelets, epithelial cells and cancer cells (3, 5, 7- 9).

Recent studies have reported the isolation and characterization of these vesicles in

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physiological fluids such as normal urine, plasma, bronchoalveolar lavage and synovial fluid (4). Exosomes are released into the extracellular space after the merging of the endosomes with the cell membrane. Early endosomes become part of the multivesicular bodies (MVBs) that undergo a maturing process that provides for a gradual change of the protein composition of the vesicles contained within; exosomes represent the intraluminal vesicles of MVBs that merge with the plasma membrane resulting in the exocytosis of the vesicles contained (10, 11). Lipid rafts or cholesterol and sphingolipid microdomains, enriched in plasma membranes, are shown to play an important role in the formation of exosomes (10).

Depending on the cell types from which they are derived, exosomes play a role in diverse physiologic and pathological processes serving as a novel and more intricate form of cell-cell communication. Tumor cells also produce exosomes, evidently abundant in culture and malignant effusions (11). Many studies have demonstrated that exosomes play a role in immune system activation during cancer progression and also in the tumor microenvironment (11). Among the proteins that can be carried by exosomes there are those belonging to the family of molecular chaperones.

Molecular chaperones, many of which belong to the Heat shock proteins (Hsps) family, are important players in protein homeostasis and cell tissue physiology, as well as in protection against stressors. They assist nascent protein in folding correctly or in refolding if partially denatured and they also drive protein translocation across membranes or, if proteins are damaged, toward degradation (12, 13). Moreover, molecular chaperones have other roles such as participation in immune system regulation (14, 15), cell differentiation (16), apoptosis and carcinogenesis (17-19). Hsps are normally considered intracellular or associated to some organelles, but many studies have shown that they can also occur in extracellular and in non-canonical sites inside the cells (20).

Exosomes have been described as potent export vehicles for Hsps from the endosomal compartment into the extracellular environment (21). For example, Hsp60, is considered a mitochondrial chaperone that is essential for the mitochondrial protein physiology (22). The range of functions attributed to Hsp60 has recently expanded and in particular it is evident that it plays a role in immune system regulation (15), apoptosis (17) and also carcinogenesis (19). Its levels have been found increased in a number of tumors (19) and it has been hypothesized that Hsp60 overexpression have an important role on cancer development and progression (19). There is increasing evidence that Hsp60 is localized on plasma membranes and also outside the cell where it mediates interactions between cells (9, 23, 24).

In recent studies it has been demonstrated that Hsp60 is released via exosomes from cardiac myocites in both basal state and following mild stress (21). Moreover we have demonstrated that human tumor cells can actively release Hsp60 by a nonconventional secretion mechanism: the lipid raft-exosome pathway (9, 23). These findings suggest a new role for extracellular Hsp60 in the cross talk between tumor cells and the immune system (23, 24).

Expression of Hsp60 on the surface of exosomes, released by tumor cells, may be considered as a danger signal for the immune system. The molecular composition of exosomes reflects the specialized functions of the original cells (25). Through their ability to bind target cells, they are likely to modulate selected cellular activities.

The presence in accessible fluids of exosomes containing biomarkers, such as Hsp60, may be useful for the early detection of a disease status. Exosomes offer huge opportunities for clinical applications that include their use as potential novel biomarkers for the diagnosis or prognosis of different diseases or for therapeutic application and drug delivery.

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