

Immunosuppressive role of extracellular vesicles: HLA-G, an important player

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Extracellular vesicles (EVs) are bilayer membrane structures composed of proteins, lipids and nucleic acids (DNA and RNA). Their specific cargo is peculiar and derives from the cell of origin. They have been involved in many physiological and pathological processes. Regarding immune biology, EVs play an important role, as they may enhance or suppress the immune system (1).

Therefore, EVs act as regulatory agents in the immune response. They are a heterogeneous population of vesicles originated from cell plasma membrane or from the endosomal compartment. EVs, independently from their origin, may remain in the extracellular space in proximity of their origin or may move into biological fluids reaching distant sites. Indeed, they have been found in plasma, milk, urine, cerebrospinal fluid, amniotic fluid and tumors (2). Cell-released EVs transfer their cargo into receiver cells, transferring information from one cell to another (3). The EVs cargo includes a variety of receptors, soluble proteins, adhesion molecules, enzymes, chemokines, and cytokines; they also contain several nucleic acid species such as mRNAs, microRNAs or long non-coding RNAs (4).

EVs have been proposed as immunotherapeutic agents as they are involved in several aspects of immunity including immune response, immune suppression, immune surveillance and antigen presentation. In particular, EVs isolated from immune cells, for example, dendritic cells (DCs) promote the immune response. At variance, tumor-derived EVs may favor immune escape (5). In this contest, EVs may act in concert with several soluble factors such as

PGE², IDO and HLA-G (6).

The important role of HLA-G and EVs was well described in a recent review (7). In this paper, the authors clearly describe the function of the secreted form of HLA-G within EVs, compared with the free soluble fraction. The secreted forms of HLA-G are mainly involved in immune tolerance in cancer and pregnancy (7). However, most of the studies do not discriminate the free soluble form from the EV associated HLA-G.

The HLA-G molecule belongs to the family of non-classical human leukocyte antigen (HLA) class I with a low polymorphism and a restricted tissue distribution. HLA-G is present in different molecular structures due to different splicing of the primary transcript. There are seven isoforms. The isoforms HLA-G1, G2, G3 and G4 are transmembrane while the isoforms G5, G6 and G7 lack the transmembrane domains and are soluble (7,8). In addition, all the membrane-expressed isoforms may be cleaved by metalloprotease enzymes and became soluble (9).

Initially, HLA-G was first described to protect the fetus versus the maternal immune system and to be involved in immune-privileged adult tissues. HLA-G has also been shown to regulate immune cells, inhibiting the activation of NKs, T lymphocytes and DCs and to favor tumor immune-escape. In the past, the immune modulatory function of circulating HLA-G was only related to its free soluble form. Riteau *et al.* first described the presence of HLA-G within EVs (HLA-G-EVs) in supernatant of melanoma cell line (HLA-G positive) (10). They found the full-length G1

isoform, usually detected only in cell membrane. It is well known that tumor cells release many factors to orchestrate their progression and EVs play a relevant role in this phenomenon. A few other groups have observed HLA-G within EVs in a tumor microenvironment (11-13). Tumor EVs stimulate angiogenesis, tumor cells growth, favor the metastatization process and induce immune escape (14). In the review of Rebmann *et al.*, the role of HLA-G-EVs in tumor immune escape is well highlighted (7). In renal cell carcinoma cells, EVs carrying HLA-G impair the differentiation process of monocytes in DCs, inhibiting T cell activation and proliferation (7,11). Moreover, the presence of HLA-G-EVs has recently been proposed as negative prognostic factor for neoadjuvant chemotherapy-treated breast cancer patients. Furthermore, increased level of HLA-G-EVs in peripheral blood has been related to circulating stem cell-like tumor cells and poor prognosis. On the contrary, high levels of soluble HLA-G in blood stream suggests a better scenario. This is the first demonstration of a differential role of soluble fractions of HLA-G, depending on encapsulation or not within EVs (7,12).

Then again, it has been known for decades that overexpression of membrane-bound and soluble HLA-G are up regulated or re-expressed in tumors (both solid and hematological) such as renal cell carcinomas, esophageal squamous cell carcinoma, colorectal cancer tissues, breast cancer, melanoma and pancreatic cancer (15). In some cases, the soluble form of HLA-G has been proposed as a diagnostic or prognostic biomarker.

Another fundamental process in which HLA-G is involved is the tolerance of maternal immune system against fetus. In the review of Rebmann *et al.*, it has been underlined that early and term placenta release exosomes containing HLA-G5 (7,16). This observation has important implications in the relationship between trophoblast and maternal immune system (17).

Although the biological activity of HLA-G is well known, the mechanism of action of HLA-G-EVs is still debated. One possibility is the interaction of HLA-G carried by EVs with specific receptors expressed by target cells. Another possibility is the direct transfer of HLA-G to target cells by the EV fusion with the cell plasma membrane. This second mechanism has been reported for T cells which may acquire HLA-G from tumor cells with consequent changes from an activated to a regulatory phenotype. Another option is the internalization of EV-carrying HLA-G with the activation of immune-modulatory intracellular pathways (7).

In conclusion, the HLA-G-bearing EVs is an important

element in HLA-G biology. Although many open questions regarding the mechanism of action remain, it is clear that EVs containing HLA-G are biologically active and are potential diagnostic biomarkers in several diseases.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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