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Titolo	GANGLIOSIDE-DEPENDENT MEMBRANE ORGANIZATION CONTROLLING THE ADHESION AND MOTILITY OF HUMAN OVARIAN CANCER CELLS [Tesi di dottorato]
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Sommario	Glycosphingolipids, due to their tendency to form laterally separated liquid-ordered phases, possess a high potential for the creation of order in biological membranes. The formation of glycosphingolipid-rich membrane domains within the membrane has profound consequences on the membrane organization at different levels, and on the conformational and biological properties of membrane-associated proteins and multimolecular protein complexes (1). Glycosphingolipids modulate several signal transduction processes controlling cell proliferation, survival, differentiation, and transformation. Since alterations in the expression of carbohydrate epitopes associated with glycosphingolipids are frequent in tumors, it has been hypothesized that glycosphingolipids could play important roles in modulating some of the properties of tumor cells. In fact, some tumors are characterized by the ability to manipulate sialylation processes determining the formation of antigenic determinants resulting from an “aberrant glycosylation” and affecting cell homeostasis, altering the normal signaling pathways (2). Thus, an ever-increasing interest to this regard is being devoted to gangliosides, sialic acid-containing glycolipids, and to the enzymes affecting sialylation. Both sialyltransferases and sialidases seem to be involved in the phenomenon of aberrant sialylation in tumor cells. The genetic (stable overexpression of sialyltransferase I - SAT-I or

GM3 synthase) or pharmacological (selective pressure by N-(4-hydroxyphenyl)retinamide) manipulation of A2780 human ovarian carcinoma cells allowed us to obtain monoclonal cells characterized by higher GM3 synthase activity respect to wild type cells (3-5). High GM3 synthase expression resulted in 1) elevated ganglioside levels, 2) reduced in vitro cell motility and increased adhesion to fibronectin, 3) enhanced expression of the membrane adaptor protein caveolin-1, an integral membrane protein playing multiple roles as negative regulator in the progression of several types of human tumors (6,7). The correlation between high ganglioside levels and decreased motility/increased adhesion were confirmed through administration of exogenous gangliosides which was not only able to strongly reduce in vitro cell motility, but also to significantly increase cell adhesive ability to fibronectin in wild type cells, low GM3 synthase expressing A2780 cells. Furthermore, in high GM3 synthase expressing clones, such as A2780/SAT-I cells, ganglioside depletion by treatment with the glucosylceramide synthase inhibitor D-PDMP was able to strongly reduce adhesion and to increase cell motility. The ?5?1 integrin, which mediates cell adhesion, is the most expressed integrin heterodimers in A2780 cells. Since fibronectin is the preferential ligand of integrin ?5?1, it has been hypothesized that this integrin heterodimer might be involved in the regulation of adhesion and motility in human ovarian carcinoma cells. On the other hand, A2780/SAT-I cells also showed an increased adhesion to laminin and vitronectin suggesting a possible role of their respective integrin receptors in the regulation of GM3-mediated adhesion. The role of a glycosphingolipid/caveolin-1 signaling complex in the negative regulation of A2780 cells motility has been reported, showing that high levels of caveolin-1 and high levels of gangliosides are necessary, but not sufficient, to down-regulate tumor cell motility (5). In GM3 synthase expressing cells, caveolin-1 and gangliosides were highly enriched in detergent-resistant membrane fractions (DRM) prepared in the presence of Triton X-100. D-PDMP treatment determined changes in the lipid distribution of several lipids in sucrose gradient fractions, and also altered protein distribution determining a shift of both caveolin-1 and c-Src, also involved in the previously mentioned complex signaling pathway, from the DRM fraction to intermediate fraction. Integrins, particularly ?5 and ?1 integrin subunits, following ganglioside depletion move from the high density fraction to DRM and intermediate fractions. D-PDMP treatment also affected protein association with caveolin-1, determining an increased association of this protein, a potential molecular organizer, with integrin subunits ?5 and ?1 without affecting the total level of proteins. The in vitro adhesion of A2780/SAT-I cells was markedly higher in caveolin-1 silenced cells compared with scramble sequence transfected cells, suggesting a leading role of caveolin-1 in the regulation of the cell adhesion signal in this cell model. On the other hand, treatment of A2780 cells with exogenous gangliosides only slightly increased the expression of caveolin-1; while it markedly increased the phosphorylation of caveolin-1 at tyrosine 14. Conversely, ganglioside depletion in high GM3 synthase-expressing clones by D-PDMP treatment markedly reduced caveolin-1 phosphorylation. These data suggest that phosphorylation of caveolin-1, rather than caveolin-1 total level, is controlled by gangliosides and is crucial in the control of tumor cell adhesion. These data suggest a novel role of gangliosides in regulating tumor cell adhesion and motility, by affecting the organization of a signaling complex organized by caveolin-1, and

	imply that GM3 synthase is a key target for the regulation of cell motility and adhesion in human ovarian carcinoma.
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