1. Record Nr. TD16001097

Titolo	ROLE OF GANGLIOSIDES IN MODULATING THE MOTILITY OF HUMAN CANCER CELLS [Tesi di dottorato]
Editore	Università degli Studi di Milano, 2013-02-19
Lingua di pubblicazione	Inglese
Formato	Tesi di dottorato
Livello bibliografico	Monografia
Note	diritti: info:eu-repo/semantics/openAccess
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. Alterations in the structures of carbohydrate epitopes associated with glycosphingolipids are a common feature of tumors and tumor cells (“aberrant glycosylation”). In particular, tumors are characterized by the peculiar ability to manipulate sialylation processes (1). This abnormal sialylation process generates peculiar antigenic determinants, which are normally absent in healthy cells, and affects cell homeostasis, altering the normal signaling pathways. Indeed, glycosphingolipids in tumor cells have been implicated in the regulation of cell adhesion, motility, recognition, survival and proliferation (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 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). Administration of exogenous gangliosides was able to strongly reduce in vitro cell motility and to increase cell adhesive ability to fibronectin in wild type cells, which are low GM3 synthase-expressing A2780 cells. Conversely, in high GM3 synthase-expressing clones, ganglioside depletion by treatment with the glucosylceramide synthase inhibitor D-PDMP was able to strongly increase cell motility and to reduce adhesion. In these cells, transient silencing of caveolin-1 by siRNA also led to increased motility. Thus, high levels of caveolin-1 and high levels of gangliosides are necessary, but not sufficient, if independent, to down-regulate tumor cell motility. Treatment of A2780 cells with exogenous gangliosides only slightly increased the expression of caveolin-1; on the other hand 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 motility. The non-receptor tyrosine kinase c-Src plays a crucial role in controlling the motility of these cells. In fact, 1) the motility of low GM3 synthase-expressing cells was reduced in the presence of a Src inhibitor; 2) c-Src was less active in high GM3 synthase-expressing clones; 3) D-PDMP treatment of high GM3 synthase-expressing cells led to c-Src activation, while gangliosides administration in wild type cells, low GM3 synthase-expressing A2780 cells reduced c-Src kinase activity. In high 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. In the presence of D-PDMP treatment, the distribution of several lipids in sucrose gradient changed, followed by a shift of both caveolin-1 and c-Src from DRM fraction to intermediate fraction. However, integrins, which are receptors that mediate attachment between cells moved from high density fraction to DRM and intermediate fraction. All of these data suggest a novel role of gangliosides in regulating tumor cell motility, by affecting the organization of a signaling complex organized by caveolin-1, responsible for Src inactivation downstream to integrin receptors, and imply that GM3 synthase is a key target for the regulation of cell motility and adhesion in human ovarian carcinoma.
Localizzazioni e accesso	http://memoria.depositolegale.it/*/http://hdl.handle.net/2434/217448