Glycomics & Lipidomics

Extended Abstract

Specific sialoglycan structures on the cell surface correlate with the ability of cancer cells to form avascular multicellular 3D tumor spheroids and in vivo xenograft tumors

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Abstract

Multicellular 3D tumor spheroid (MTS) formation in cancer research has been designed to mimic tumor-like developmental patterns in vitro. Tumor growth and invasion is known to be highly influenced by aberrant cell surface-specific sialoglycans on cell surface glycoproteins. Aberrant sialoglycan patterns that facilitate MTS formation has not been well defined. To evaluate the role of sialylation of cancer cell surfaces in spheroid formation, we used the cyclo-RGDfK(TPP) approach to biochemically induce cell aggregation and compaction, transmogrifying monolayer cancer cells into tumor spheroids. The cyclo-RGDfK(TPP) peptide-based platform causes specific biochemical alterations of cell surface receptors inducing self-assembly in monolayer cell cultures into 3D MTS by facilitating cell-cell recognitions, interactions and adhesion. Matrix-free spheroids from breast MCF-7 and pancreatic PANC1 cancer cell lines and their respective tamoxifen (TMX) and gemcitabine (Gem) resistant variants formed tight spheroids while all PANC1 cells formed loose aggregates. MCF-7 and PANC1 cells and their drug-resistant variants expressed different sialic acid (SA) content on their cell surfaces. α2,3- and α2,6-sialic acid surface residues facilitated spheroid formation under cyclo-RGDfK(TPP)-induced self-assembly. Pretreatment with α2,3-SA specific *Maackia amurensis* (MAL-II) lectin, α2,6-SA specific *Sambucus nigra* (SNA) lectin, and exogenous α2,6-SA specific neuraminidase (*Vibrio cholerae*) dose dependently reduced spheroid volume. Oseltamivir phosphate (OP) treatment enhanced cell aggregation and compaction forming spheroids. PANC1 and MDA-MB231 xenograft tumors from untreated and OP-treated RAGxCγdouble mutant mice expressed significantly higher levels of α2,3-SA over a-2,6-SA. The present report provides evidence for the important role of specific sialoglycan structures expressed on cancer cells to form avascular multicellular tumor spheroids and in vivo xenograft tumors. Future studies should build upon these findings and explore alternate and novel methods to target the cancer cell glycome and the unique sialylation patterns of the adhesion molecules involved in spheroid formation and tumor progression.

The multicellular tumor spheroid (MTS) is a promising 3D model platform that enables the study of tumor cell development, morphology, cellular motility and drug resistance *in vitro*. The MTS mimics the *in vivo* microenvironment which plays a dominant role in multidrug resistance and various cell processes, including epithelial-mesenchymal transition (EMT) and metastasis. MTSs are generally used for novel anticancer drug screening. Since spheroids resemble the 3D architecture of avascular tumors, including multicellular arrangement and extracellular matrix deposition typically found *in vivo*, spheroid cells also demonstrate enhanced resistance to chemotherapy. Tumor spheroids in matrigel or in ECM-based matrixes are good study models to investigate cell motility and anti-metastatic compounds *in vitro*. However, novel MTS formations, particularly under matrix-free conditions, are being developed to study the 3D architecture of avascular tumor models, especially in relation to metastasis, invasion and therapeutic drug screening. Presently, the molecular development of MTS formation by cancer cells may involve (a) cell surface proteins binding fibronectin which induces 3D cohesion, (b) under conditions of random positioning machine (RPM) simulating microgravity, the expression of 28 genes aside from β-tubulin is mutually controlled by a key cytokine interleukin-8 (IL-8 or CXCL8) gene within the framework of 6 extracellular, 6 membrane, 15 cytoplasmic and 2 nuclear proteins, and/or (c) the integrins' interactions with the extracellular matrices (ECM) and intracellular components within the cellular cytoskeleton in particular response to mechanical stimulatio. It has been reported that MTS formation involves a number of highly glycosylated integrins such as αvβ3 and α5β1 on the cell surface. It is well known that integrin expression correlates with metastases in a large variety of cancers. Since integrins are highly glycosylated receptors, recent reports have reviewed altered expression of sialylated glycoproteins with elevated levels of cell-surface α2,6-sialic acids (SA) that are linked to colorectal cancer metastasis, radio-resistance, and chemoresistance. In addition, the altered mammalian

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sialidase(s) expression was reported not to result from metastatic potential, but rather from a determining event affecting metastatic ability. It was proposed by the report that SA expression on tumor cell surfaces appears to vary from cell to cell. Other reports have shown that altered sialylation of glycoproteins is closely associated with metastatic potential and cell invasiveness. With regard to integrins, Pochećproposed that the β1-6-branched sialic acid of αvβ3 integrins promotes the metastatic characteristics and migration of melanoma cells. Recently, we have shown that a synthetic cyclic RGD-peptide induces formation of 3D MTS in a simple, single-step, reproducible procedure. The resulting MTS can be developed and employed as 3D models for assessing antitumor drug efficacy and was studied in twelve cancer cell lines. The report describes the self-assembly of cancer cells from monolayer cultures into MTS, a process that was directly induced by the RGD-peptide. The self-assembly formation of monolayer cultures into MTS was induced by the cyclic Arg-Gly-Asp-D-Phe-Lys (cyclo-RGDfK) peptide, modified with 4-carboxybutyl-triphenylphosphonium bromide cation (TPP). The resulting modified peptide, cyclo-RGDfK(TPP) was used in the concentration range of 10-100 uM. The 3D characterization of the spheroids showed unimodal structures, ranging from 60-120 µm in diameter, and varying between cell lines and medium serum concentration. The report also proposes that these cyclo-RGDfK(TPP) peptides mimick the natural ECM protein's ability to induce cell aggregation via α5β1 integrin. To evaluate the role of sialylation of cancer cell surfaces in spheroid formation, we used the cyclo-RGDfK(TPP) approach to biochemically induce cell aggregation and compaction, transmogrifying monolayer cancer cells into tumor spheroids.

Biography

Dr. Szewczuk is Full Professor of Immunology and Medicine, Queen's University, Kingston, Ontario Canada. Dr. Szewczuk's current research is focusing on the role of glycosylation in receptor activation with a particular focus on alternate new active tumor targeting drug delivery systems.

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