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The new general biological property of stem-like tumor cells

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Motivation and Aim: Previously, we have demonstrated poorly differentiated cells of various origin and genesis, including tumor stem-like ones, to be capable of native, i.e. without using any artificial compounds or procedures, internalizing extracellular double-stranded DNA (dsDNA) fragments. To detect and investigate the cells in question, we have designed the 500 bp dsDNA probe, which is TAMRA-labeled PCR-amplified human *Alu*-repeat (*Alu*-TAMRA DNA probe) [1]. *In vivo* engraftment and transcriptome analysis have revealed both tumor-initiating properties of DNA-internalizing cells of murine Krebs-2 carcinoma and overexpression in these cells of genes responsible for asymmetric division, pluripotency, epithelial-to-mesenchymal transition, drug resistance etc. [2]. In experiments with other tumor models (primary human glioblastoma cell lines, established U87 human glioblastoma cell line, Epstein-Barr Virus-induced B-lymphoma (EBV+ B-lymphoma), murine hepatocarcinoma and Lewis sarcoma), TAMRA+ cells have also been proved to manifest the properties characteristic of tumor stem-like cells (TSCs). It is these cells that are essential for the development of grafts *in vivo* and are the center of spheres formation due to secretion of a set of cyto- and chemokines [3-6]. However, the exact mechanism of extracellular dsDNA internalization into cells has not yet been clarified.
Methods and Algorithms: In the current report, for murine Krebs-2 carcinoma and human EBV+ B-lymphoma, there have been determined the mechanisms mediating the interaction of dsDNA with the surface of TSCs and its consequent internalization into these cells. The following approaches were used: (i) electrophoretic separation of cells in a free volume with the following estimation of the number of cells capable of binding TAMRA-dsDNA; (ii) pre-exposure of cells to various proteases and/or compounds inhibiting pino- and endocytosis, followed by assessing the resulting efficiency of *Alu*-TAMRA DNA probe internalization; (iii) analysis of the transcriptome of the indicated cell lines followed by the qPCR to validate the differences in expression of some target genes identified as possible participants in the process of dsDNA internalization.
Results: It was shown, that the process of binding and internalizing is rather complicated and composed of the following successive stages: (i) initiating electrostatic interaction and contact of a negatively charged dsDNA molecule with a positively charged molecule(s) on the surface of a TSC; (ii) binding of the dsDNA probe to a tumor stem cell surface protein(s) via the formation of a strong chemical/molecular bond; (iii) the very internalization of dsDNA into the cell. Binding of DNA to cell surface proteins is determined by the presence of heparin/polyanion-binding sites within the protein structure, which can be competitively blocked by heparin and/or dextran sulfate, wherein heparin blocks only the binding, while dextran sulfate abrogates both binding and

internalization. The abrogation of internalization by dextran sulfate implies the role of scavenger receptors in this process. Cells were shown to uptake DNA in amounts constituting ~0.008 % of the haploid genome. Inhibitors of caveolae-dependent internalization abrogate the DNA uptake in Krebs-2 cells, and inhibitors of clathrin/caveolar mechanism block the internalization in EBV+ B-lymphoma cells.
Conclusion: It is shown for the first time that, in contrast to the majority of committed tumors cells, stem-like tumor cells of Krebs-2 and EBV+ B-lymphoma carry a general positive charge on their surface. It turns out that both proteoglycans/glycoproteins and complexes of scavenger receptors/glycosylphosphoinositol-associated proteins with caveolae or clathrin vesicles are the basic factors determining the interaction and internalization of dsDNA into TSCs. Thus, the basic provisions of the concept characterizing the principle of the organization of the stem-like tumor (and, possibly, some other poorly differentiated) cell surface factors to be formulated as a concept of "group-specific cell surface factors, which form the profile of tumor stem-like cells". The results of bioinformatic and experimental research confirming the validity of the proposed concept are presented.
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