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The Content Analysis of ALDH$^+$ and SORE6$^+$ Populations of Cells in Cellular Lines of Thriple Negative Breast Cancer

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Abstract

Existence of the special cancer stem cells (CSC) possessing of raised tumor - initiating potential and of chemotherapeutic medicines resistance, finds more and more confirmations. Nevertheless, correct detection of subpopulation of CSC for each type of an oncological disease remains a big problem. For today are established several markers of RSK at the breast cancer (BC), however whether these signs characterize the same population of cells remain unclear. In the carried-out work we detected CSC in lines of thriple negative BC (TNBC) and in one line of a glioblastoma on activity of aldehyde dehydrogenase (ALDH) and with use of a reporter construct of SORE6. We found lack of correlations in percentage of ALDH$^+$ and SORE6$^+$ populations of cells for the majority of TNBC lines and for a glioblastoma.

Keywords: cancer stem cells, thriple negative breast cancer, glioblastoma, aldehyde dehydrogenase, reporter of SORE6.

INTRODUCTION

The tumor represents heterogeneous cellular population in which are allocated more differentiated cells forming the tumor bulk, and a quantity of cells possessing of stem cells properties [1]. The Cancer Stem Cells (CSC) are characterized by the increased clonogenic activity and when injected to immunodeficient mice initiate development of malignant new growths. The understanding of the nature of CSC has fundamental value both for interpretation of processes of carcinogenesis, and for improvement of therapy of oncological diseases. CSC is detected, as a rule, on an expression of one or several certain superficial markers, and for each type of tissues this set is unuque. Nevertheless, even at rather reliable markers of CSC, it is impossible to strictly separate the tumor - initiating cells from the cells which do not have such potential.

CSC at the breast cancer (BC) for the first time was identified as CD44$^+$/CD24$^{-/low}$ [2].

It was established a bit later that cancer cells with the raised expression of intracellular enzyme aldehyde dehydrogenase 1 (ALDH1) also possess tumor - producing properties [3]. ALDH1 oxidizes aldehydes to carbonic acids, and it is considered that its metabolic activity can serve as one of the factors of stability of cancer cells to chemotherapeutic medicines [4,5]. In neuronal and haematopoietic stem cells ALDH1 transfers retinal to retinoic acid, which through activation of its own nuclear receptors, serves as the important regulator of a cellular differentiation [6,7]. Today the analysis through ALDEFLUOR is widely applied to a research of activity of enzyme. ALDEFLUOR is a method that was initially developed for selection of haematopoietic stem cells [8]. Aldefluor-reagent represents molecules of the aminoacetaldehyde linked with fluorochrome. Thanks to lack of a charge a reagent is freely gets through a native membrane of living cells and under the influence of an aldehyde dehydrogenase turns into aminoacetate which is negatively charged and cannot get from a cell to the outside. Thus, cells with active aldehyde dehydrogenase accumulate a fluorescent tag and can be sorted on FACS.

Nevertheless, crossing of subpopulations of CD44$^+$/CD24$^{-/low}$ and ALDH1$^+$ on these signs turns out to be extremely low what leads to need of search of more exact and consistent ways of selection of CSC [9-12]. So, Tang with colleagues developed the lentivirus reporter (SORE6 reporter) in whom the destabilized fluorescent protein mCherry is expressing under the control of the promotor that is dependent on trancriptional factors Oct4 and sox2 - two main TF, that are active in CSC [13]. Using this method, researchers successfully managed to allocate cells fraction from several BC lines and samples of patients which was characterized by the high potential of tumor producing and by chemotherapeutic medicines resistance. It is interesting that percentage of such CSC in tumoral culture was as higher, as the line was considered to be more malignant.

In our work we decided to investigate the maintenance of CSC in various lines of thriple negative breast cancer and in one line of a glioblastoma, using both methods of detection - Aldefluor and SORE6 dsmCherry reporterny system. Glioblastoma is known as extremely malignant tumor which is difficult to treat. It is known that the glioblastoma contains in the structure many types of cells [14]. Thriple negative breast cancer (TNBC) represents heterogeneous group of neolpasms of a mammary gland and also is characterized by the aggressive course of a disease and low general survival. Lack of the known therapeutic targets interferes with development of target

therapy of TNBC, therefore research of BC in TNBC is perspective from the point of view of development of such targets.

MATERIALS AND METHODS

In this work were used cellular lines of the American typical collection of cultures (ATCC®).13 lines of TNBC HCC1937, HCC1143, MDA-MB-468, HCC38, HCC70, HCC1806, BT-549, Hs 578T, MDA-MB-231, MDA-MB-436, MDA-MB-157, MDA-MB-453, HCC1395 and one cellular line of a glioblastoma U-118MG were cultivated at 37 degrees in 5% of CO_2- incubator in the RPMI1640 medium or DMEM with addition of 10% of FBS and with antibiotics penicillin/streptomycin.

Research of activity of aldehyde dehydrogenase

BODIPY- aminoacetalaldehyde diethyl acetal (Cayman chemical) was incubated in 2M HCl (1:1) solution within 2 hours in order to transfer reagent to active BODIPY-aminoacetalaldehyde.

The buffer for staining was prepared on the basis of the phosphatic and salt buffer with addition of verapamil to final concentration 50 μMol. As a negative control for staining in the buffer was added N, N-dietilaminobenzaldehyde (DEAB) to the final concentration of 0.15 mMol.

Then in the solution was added BODIPY-aminoacetaldehyde (a drain of 125 μMol) in dilution from 1/10 to 1/250, cultures of cells were washed in order to remove the medium and after that was poured the ready buffer. After a half-hour incubation the buffer was deleted, PBS was added and was estimated the ALDH activity on a sorter (FACSVantage SE, Bekton Dickinson).

Assembly of the lentivirus reporter and transduction of cells

Assembly of the lentivirus reporter SORE6+ dsmCherry was carried out according to the description of the procedure in original article. In work we used pPACK1 Lentiviral Vector Packaging Kit (Systems Biosciences) which allowed receiving a high titer of a lentivirus construct. Efficiency of infection made> 90% without application of a polibren and did not require the subsequent rounds of selection. Detection of SORE6+ cells was carried out by means of a sorter and a fluorescent microscope.

Statistical analysis

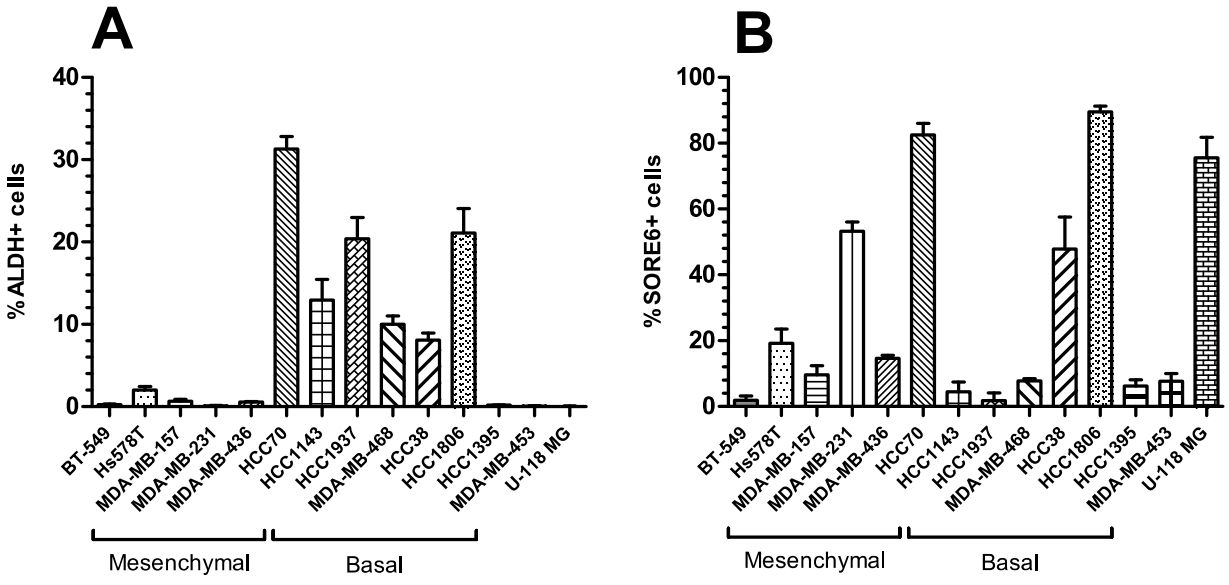
Calculation of results of a research was carried out with use of the GraphPad Prism 5 program for Windows. In the analysis of data the exact test of Fischer was used.

RESULTS

The majority of the studied cellular lines contain ALDH+ fraction of cells.

We conducted a research of contnt of ALDH+ of population of cells in 13 lines of TNBC and in 1 line - glioblastoma (fig. 1). As a result it was established that 11 of 13 TNBC cellular lines contain ALDH+ fraction of cells, which sizes ranges from 0.2% to 31% depending on the line (fig. 1A). In two lines of cells of TNBC MDA-MB-231 and MDA-MB-453 and in the line of a glioblastoma U-118 MG it was not succeeded to detect ALDH+ signal.

To check the existence of correlation between TNBC subtype and ther size of ALDH+ fraction we divided all TNBC explored lines into two conditional groups - ALDH -/low (0-5%) and ALDH high (5-31%). It was revealed that all 5 lines of a mesenchymal subtype are characterized by absence or by low size of ALDH+ fraction while 6 of 6 lines of a basal subtype contain a significant amount of ALDH+ cells (P=0.0022, the exact test of Fischer).



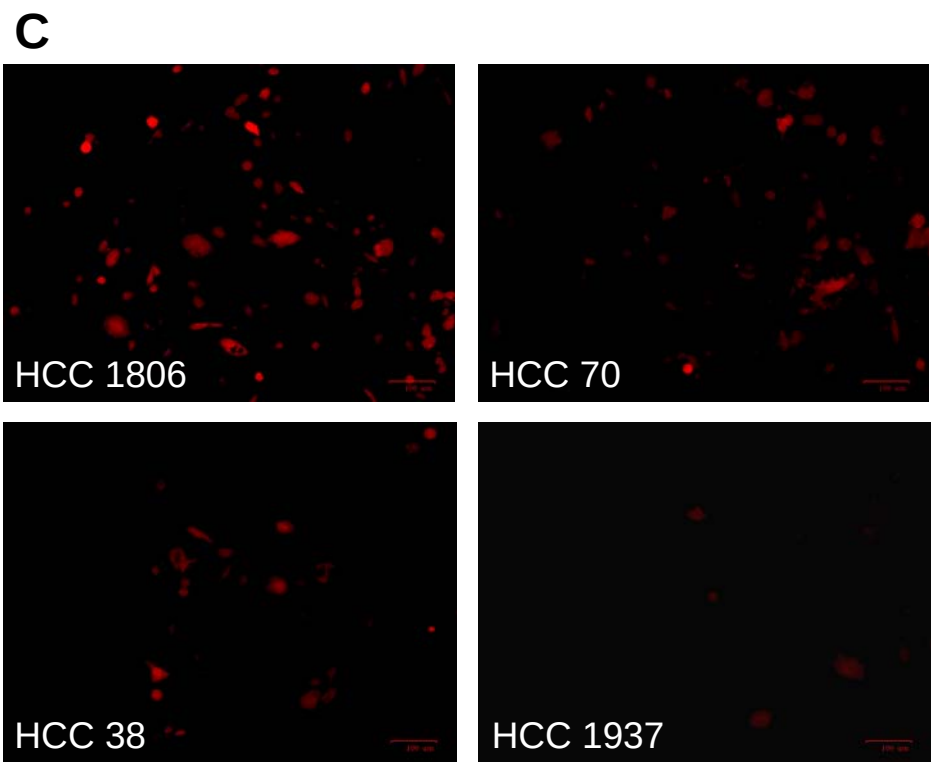


Fig. 1. The maintenance of cancer stem cells in various lines of thriple negative breast cancer and in one line of glioblastoma (U-118 MG). A. Persentage of ALDH+PCK received as a result of FACS analysis. Results represent an arithmetic average + SD, received during 3 independent experiments (n=3). B. Persentage of SORE6+ PCK, received as a result of FACS of the analysis. Results represent an arithmetic average + SD (n=3).

All studied cellular lines contain SORE6+ fraction of cells.

13 TNBC lines and 1 line of glioblastoma were transduced with high efficiency by lentivirus reporter SORE6 dsmCherry. FACS analysis showed that all cellular lines contain SORE6+ population of cells which sizes are ranging from 1.9% to 90%. Associations between TNBC subtype and the size of SORE6+ population were not established. In the U-118 MG line about 75% of population were SORE6 positive.

SORE6+ population quantitatively surpasses ALDH+ population of cells in the majority of cellular lines.

In 10 of 13 TNBC cellular lines the percentage ratio of SORE6+ fraction and ALDH+ differed more, than twice. SORE6 + population is more than ALDH+ of population of cells. In the MDA-MB-231 and HCC1143 lines prevails ALDH+ fraction of cells whereas in MDA-MB-468 percentage of ALDH+ and SORE6 + cells differed slightly (10.3 and 7.76, respectively). In the U-118 MG line were not revealed ALDH+ cells, while 75% of population were detected as SORE6+.

DISCUSSION

The concept of existence of the special cells in tumoral mass that are having properties of stem has a fundamental value for understanding of processes of carcinogenesis, metastasis and resistance to chemotherapy [15-17]. In recent years more and more studies are carring out, that are aimed to search and characterise cancer stem cells, both in the established tumoral cellular lines and in resectional material of patients with various oncopathologies including those with breast cancer [11,18,19].

In the conducted research it was established that the majority of the TNBC cellular lines contain ALDH+ population of cells. However the size of ALDH+ cells fractions strongly varies depending on the line. The greatest percent of ALDH+ of cells was established in cellular lines which according to the classification of university of Vanderbildt, are refered to a basal subtype [20,21]. The high expression of components of a cellular cycle and the increased proliferative potential is characteristic of a basal subtype [21]. Unexpectedly it was revealed that the smallest ratio of ALDH+ cells to the total number of cells in culture (0-2%) is characteristic for those cells lines, which are refered to a mesenchymal subtype according to the same classification. Signs of mesenchymal epythelial transitions, the increased maintenance of components of signaling ways of cellular mobility and growth factors are highly characteristic of this subtype [21]. For a part of cellular lines of a mesenchymal subtype was shown the high expression of the genes associated with stem cells and a low expression of claudins 3, 4 and 7 [22,23]. It is obvious that the low persentage of ALDH+ cells detected by us in cellular lines of a mesenchymal subtype will not be coordinated with the increased stem properties which were established as characteristic of a subtype of these lines. Low persentage of ALDH+ cells in lines of MDA-MB-231, MDA-MB-157, MDA-MB-436, BT549 and HS578T was

also shown in other research [24]. Similar reverse correlation can testify to ambiguity of use of ALDH+ sign as a strict marker of stem cells at TNBC. A question about to which extent it is possible to trust data, received with the use of cellular lines, is still unclear. So, in recent work on classification of TNBC lines it was established that the profile of an expression of 9 TNBC lines, among which there were MDA-MB-231, MDA-MB-157, MDA-MB-436, BT549 and HS578T is not characteristic both for normal cells of a mammary gland, and for cancer, and practically is never observed in vivo [25]. Apparently, it can be assosiated with inevitable changes which undergo cellular lines during many years of cultivation, and with initial uniqueness of those tumors from which these lines were established. Authors warn against use of such lines, because the relevance of results can be ambiguous for the practical application.

The action of SORE6+ reporter is based on the activity of transcription factors Sox2 and Oct4, the main TF, that are regulating mechanisms of self-updating and a pluripotential of embryonic stem cells [26]. Such mediated measurement of activity of Sox2 and Oct4 with some assumption can be considered more likely showing the rectilinear and universal proof of stem properties of a cell, compare with the previous use of superficial markers or activity of aldegiddehydrogenase. According to the results received during the conducted research, all TNBC cellular lines contain population of cells with active transcriptional factors Oct4 and Sox6 that is peculiar to stem cells, in various ratios depending on the line. Dependences between a subtype of cellular lines and a quantity of SORE6+ cells were not revealed.

CONCLUSIONS

In our work we did not find any significant correlation between the maintenance of ALDH+ and SORE6+ populations of cells in TNBC lines. In the majority of the studied cellular lines the SORE6+ cells were rather higher in a percentage ratio, than ALDH+. In the line of glioblastoma U-118 MG were not detected ALDH+ cells, while the SORE6+ fraction of cells was extraordinary high**.** As far as whether these populations are blocked and whether they posess of a comparable tumor - initiating potential remains a question for further researches.

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