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Direct Cell-Cell Communication Controls the Division of the PC-3 Human Prostate Cancer Cell Line's Stem Cells

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Abstract

Disease undifferentiated cells (CSCs) are a subpopulation that can drive repeat and metastasis. In this way, treatments focusing on CSCs are required. The precise molecular mechanism by which non-CSCs regulate CSC proliferation and differentiation in the tumor microenvironment is largely unknown, despite previous findings suggesting this. In the PC-3 human prostate cancer cell line, we discovered that a direct interaction between CSCs and non-CSCs decreased CSC division [1]. When non-CSC-rich parental PC-3 cells were present in a culture, the proliferation of PC-3-derived CSCs (PrSCs) was significantly lower (47%) than when they were absent. When PrSCs were indirectly cocultured with PC-3 cells across a Transwell insert, there were no differences in PrSC proliferation, and PrSCs that were transiently bound to immobilized PC-3 cells proliferated more slowly than PrSCs. The recurrence of cell division with earlier PrSC contact was 2.8 times higher in the PrSC monoculture contrasted and that in the coculture with PC-3 cells [2]. A cell proximity assay revealed that the PrSCs were approximately 1.3 times more closely associated in the monoculture than in the coculture with PC-3 cells. In the coculture with PC-3 cells, the frequency of asymmetric PrSC division was 1.0%, while it was 6.5% in the monoculture (P 0.045). We discovered that PrSC–non-CSC contact regulates PrSC division frequency and mode through data analysis. The treatment of cancer may have a useful target in this regulation [3].

Keywords: Prostate cancer; Cancer stem cells; Tumor microenvironment; Division mode

Introduction

A subset of cancer stem cells known as CSCs are crucial to the development of malignant tumors. In spite of the fact that CSCs exist in tiny numbers in growth tissues, their capacities to self-recharge by means of symmetric division and to foster more-separated cell aggregates (e.g., non-CSCs) by means of deviated division empower them to drive the spread of disease, including through the development of a progressive construction of heterogeneous disease cells. Non-CSC cells, stromal cells like fibroblasts, immune and vascular endothelial cells, and extracellular matrix components make up the tumor microenvironment (TME), which includes CSCs. In this niche, CSCs interact with these cells to create and maintain intratumor heterogeneity. CSCs exhibit high plasticity and can dynamically switch between non-CSC and CSC states through reversible differentiation, in addition to their interactions in the TME. However, little is known about the molecular mechanisms that underlie these processes [4].

Non-CSCs that are derived from CSCs through asymmetric division play a particularly important role in controlling CSC proliferation in the TME. Maintaining intratumor heterogeneity requires interaction between the two subpopulations. Using mathematical simulation models, researchers discovered that a mechanism involving the regulation of CSC division by nonmalignant cells, including non-CSCs, keeps the number of CSCs in a tumor constant [5]. However, the precise molecular mechanisms by which CSC populations are maintained and CSC proliferation is controlled by surrounding cells are largely unknown. A depiction of these instruments could add to the improvement of novel malignant growth treatments [6].

Materials and Methods

Cell culture

The JCRB cell bank (National Institutes of Biomedical Innovation, Health, and Nutrition, Tokyo, Japan) provided us with the PC-3 human prostate cancer cell line. We used RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) to grow the cells. The medium contained 10% fetal ox-like serum (CosmoBio, Tokyo, Japan) and 1% penicillin/streptomycin (Sigma-Aldrich), and we utilized it to culture the cells at 37 °C, under a 95% air and 5% CO_2 humidified environment. PC-3 cells were transfected with a pCruz GFP expression vector (Santa Cruz Biotechnology, TX, USA) using Lipofectamine 3000 (Life Technologies, CA, USA) to produce the green fluorescent protein (g-PC3). We chose the g-PC3s by giving them G418 (100 g/mL) every day for two weeks.

Isolation of holoclones and GFP-expressing holoclones by limiting dilution

Following H. Li et al.'s method, we used PC-3's morphological characteristics to distinguish holoclones from other cell types. Using a trypsin–EDTA solution from Sigma-Aldrich, we removed the PC-3 cells from the dishes and seeded them into a 96-well plate at a density of one cell per well in 100 L of medium per well. We marked the wells with one cell each after the cells were grown overnight. We characterized individual provinces emerging from a solitary cell inside 14-20 days of culture as holo-, mero-, or paraclones, in view of their morphology [7]. The resulting holoclones were grown and transferred to 6-well plates, where they were maintained until they were nearly confluent. We put away frozen holoclones separated from PC-3 and g-PC3 cells (assigned PrSCs and g-PrSCs, individually) at −80 °C until use.

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Immunofluorescence staining

We seeded cells into a 24-well culture plate at a density of 104 cells per well. After 24 h, we fixed the cells with 4% paraformaldehyde for 15 min at room temperature, and afterward washed them with PBS (43 mM Na2HPO4, 15 mM KH2PO4, 137 mM NaCl, and 27 mM KCl; pH 7.4). The cells were blocked for 30 minutes with PBS containing 1% BSA, incubated with anti-CD44 antibody, and conjugated for 30 minutes at room temperature with PE-Cy5 (TOMBO Biosciences, Osaka, Japan) in the dark. Using a fluorescence microscope (type BZ-X700, KEYENCE, Osaka, Japan), we took immunofluorescence images after we had washed them three times with PBS [8].

Cell proximity assay

We used a MarkerGene Cell Proximity Assay Kit (Abcam, Cambridge, UK) to conduct a cell proximity assay to examine the impact of cell–cell contact on CSC proliferation. The turnover of a luminescent substrate is the foundation of this assay system, which is mediated by interactions between the two cells of interest. Each cell was transfected with both the LacZ and luc genes. As a measure of cell proximity and interaction, we measured the light emission produced by the turnover of the substrate. Using Lipofectamine 3000, we transformed the luc and lacZnls12co expression vectors, pDC57 (Abcam) and pCMVBeta (Abcam), into PrSCs to produce PrSCs carrying luc (PrSC-luc) and lacZ (PrSC-lacZ). In a 96-well black plate containing RPMI-1640 complete medium, we seeded 1 104 cells consisting of PC-3 and PrSC-luc and -lacZ (2:1:1 in terms of cell number) or PrSC and PrSC-luc and -lacZ (2:1:1 in terms of cell number). We replaced the medium with 50 L of complete RPMI-1640 medium, supplemented with 20 mM HEPES and 50 L of the Lugal substrate (1-O-galactopyranosyl-luciferin;) after incubating them for 24 or 44 h. 1.5 mg/mL). Using an Enspire multimode plate reader (PerkinElmer, Waltham, MA, USA), we measured the chemiluminescence every 5 minutes for a total of 90 minutes [9].

Results

Isolation and characterization of PC-3-derived CSCs (PrSCs)

To confine a CSC populace from PC-3 cells, we utilized a formerly settled technique with minor changes. We discovered that the resulting holoclone accounted for 9.9% of the original PC-3 cell population, which is consistent with the findings of the previous study. We used RT-qPCR to measure the levels of the CSC markers CD44, 133, and ALDH1A, which were significantly higher in the holoclone than in the original PC-3 cell population, to confirm the CSC characteristics of the holoclone. The sphere-formation assay's findings further demonstrated the PC-3 holoclone's stemness [10]. As a result, we utilized PC-3 holoclone as PrSCs for the prostate.

A direct cell–cell interaction regulates CSC proliferation

We evaluated PrSC proliferation in coculture systems to test our hypothesis that non-CSCs control CSC growth. We isolated holoclones (g-PrSCs) from PC-3 cells that were stably expressing GFP (g-PC3) in order to obtain an accurate measurement of PrSCs. We cultured g-PrSCs either with or without PC-3 cells that were more than 90% non-CSCs. When compared to the g-PrSC monoculture, the coculture with PC-3 significantly reduced g-PrSC proliferation. In addition, glutaraldehyde-fixed PC-3 cells outperformed fixed PrSCs in terms of the rate of g-PrSC proliferation. We noticed no massive change in the development of g-PrSCs when we in a roundabout way cocultured them with PC-3 across a Transwell embed. In a similar vein, when compared to the incubation in the PrSC-derived conditioned medium,

we observed a slight decrease in gPrSC proliferation of less than 10% 48 and 72 hours later. 1c) [11].

Discussion

CSCs are a subpopulation of cancer cells that can rise to the top of the hierarchical structure of heterogeneous cancer cells to initiate tumor progression. Because mathematical simulation models have suggested a regulatory role for non-CSCs in the proliferation and differentiation of CSCs, numerous previous studies have focused on the interaction between CSCs and non-CSCs in tumor progression. The regulation's precise molecular mechanism is, however, unknown. CSC markers are fluidly communicated relying upon the beginning of the cancer. CSC-like clones were isolated from PC-3 cells in this study [12]. We demonstrated that there are three distinct cell subpopulations in PC-3 cells: paraclones, holoclones, and meroclones 1A). Holoclones can be used as CSC models because they have the potential to proliferate over time. The frequency of holoclone occurrence in our study was comparable to that found in the literature. CSC markers like CD44 and 133, as well as ALDH1A1, are highly expressed in the obtained holoclone, which is designated PrSC. When compared to PC-3 cells, PrSCs have a higher activity in the formation of spheres. We argue that PrSCs are a CSC subpopulation in light of the data [13].

The underlying molecular mechanisms underlying the regulation of PrSCs remain unknown, despite the fact that our findings in the study provide insight into the role of non-CSCs in the regulation of PrSCs in terms of the frequency and mode of cell division. Both the recurrence and method of cell division in PrSCs are controlled by actual collaborations between the cell-surface atoms of PrSCs and non-CSCs, on the grounds that the two cycles are impacted after cell contact. There are two potential mechanisms for controlling these processes: 1) Non-CSCs compete with PrSCs for the essential homologous binding, and PrSC–PrSC interactions increase the frequency of division and promote symmetric division. 2) Non-CSCs simply bind to PrSCs to promote asymmetric division and reduce the frequency of division. The responsible surface molecule for the subsequent cell–cell contact signaling process must be identified through additional research. In addition, it is important to note that our observations were restricted to PC-3 cells, and it is necessary to conduct additional experiments with various cancer cells before we can draw a generalization regarding the PC-3 cell line [14].

Conclusions

The consequences of our review propose that communications among CSCs and non-CSCs direct the recurrence and method of CSC division. Our outcomes are reliable with the possibility that non-CSCs, as a part of the TME, can facilitate growth movement and heterogeneity relying upon the natural circumstances. A potential target for future pharmacological intervention in the treatment of prostate cancer might be a cell–cell-contact-dependent regulation of PrSC division.

Conflict of Interest

None

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