International Journal of Inflammation, Cancer and Integrative Therapy

Case Study **Open Access**

A Comparison of the Ability of Three Different Cancer Stem Cell Cultures to Spread

Mansaru Seno*

Department of Biotechnology and Drug Discovery, Graduate School of Interdisciplinary Science and Engineering in Health Systems, Okayama University, Okayama, Japan

Abstract

One of the main causes of cancer deaths is metastasis. In any case, the systems of disease cells gaining forcefulness and metastatic potential are still being scrutinized. Despite the fact that cancer stem cells (CSCs) have been proposed as the cells that start tumors and are responsible for cancer metastasis, no sufficiently practical models have been discovered due to the lack of CSCs. Using conditioned media (CM) from various cancer cell lines and mouse induced pluripotent stem cells (miPSCs), we have created novel CSC models. Here, we attempted to lay out the models of metastasis utilizing three unique CSC models, miPS-LLCev, miPS-BT549cm and miPS-Huh7cm cells. By injecting mice intraperitoneally, these CSC models' metastatic capabilities were evaluated. Histological examination of the developed metastases revealed differences in gene expression between the parent and metastasized cells. The outflow of CSC and stemness markers was kept up with in the cells confined from metastasis. RNA-sequencing was used to further investigate the three types of CSCs and identify the enriched cytoplasmic signaling pathways. Consequently, the three CSC models displayed distinct patterns of metastases, with miPS-LLCev cell metastasis appearing to be the most aggressive, demonstrating pulmonary metastasis and hepatocellular carcinoma, which originated within the liver. In miPS-LLCev and miPS-Huh7cm cells, the "HIF-1 pathway" was suggested as a candidate for an enriched pathway, indicating that these CSC models had distinct metastatic potential. As a result, we came to the conclusion that the CSC models developed in this study would provide accurate models of aggressiveness-related metastasis.

Keywords: Cancer; Cancer stem cells (CSCs); Metastasis; Extracellular vesicles (EVs); Microenvironments

Introduction

In the beyond twenty years, disease research has seen a few critical achievements to control the rising threat of malignant growth. Although our comprehension of the molecular and cellular mechanisms that drive cancer progression has improved, we still do not know how tumors invade other tissues and evade therapies. Phenotypic heterogeneity is the variety of functional properties and expressions of various lineage markers that tumor cells can take on as the disease progresses [1]. The intra- and inter-tumoral heterogeneity, which refers to the molecular differences brought about by tumor initiation in the same organ, can be used to classify tumors as well as biologically distinct disease entities. Cancer stem cells, or CSCs for short, are a distinct subpopulation of cells that are responsible for tumorigenesis. These cells are capable of self-renewal and differentiation, which helps to maintain the malignant growth and even encourage the invasion of adjacent tissues. CSCs are in charge of the new generation as well as those with differentiated phenotypes, whereas CSCs are in charge of the progeny and cancer-associated cells in the tumor. The question of where the CSCs came from remains unanswered [2].

The CSC niche, which CSCs can create, contains variant cellular components like cell-mediated adhesion and soluble factors, as well as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and tumor-associated neutrophils. The cells that make up the CSC niche play important roles in supporting CSC survival, such as encouraging angiogenesis, which leads to invasion and metastasis.

A novel CSC model was created by our group using mouse induced pluripotent stem (miPS) cells that were cultured in a conditioned medium (CM) that was enriched with factors related to inflammation from cancer cells [3]. We propose that the normal stem or progenitor cells found in all tissues affected by the chronic inflammation microenvironment, also known as the "cancer-inducing niche," could be the source of CSCs. Using CM from various cancer cell lines and this model, we produced CSC models. The CM of the liver cancer cell line Huh7 cells, the CM of the breast cancer cell line BT549 cells, and the tumor-derived extracellular vesicles (tEVs) of Lewis lung carcinoma (LLC) cells were used to turn miPS cells into various CSCs. The developed CSCs were capable of metastatic spread and malignant tumorigenicity. As a heterogeneous tumor component, CSCs have the potential to differentiate into vascular endothelial cells, CAFs, and TAMs, creating a niche that supports the balance between self-renewal and differentiation [4].

Materials and methods

Cell culture

In the beyond twenty years, disease research has seen a few critical achievements to control the rising threat of malignant growth. Although our comprehension of the molecular and cellular mechanisms that drive cancer progression has improved, we still do not know how tumors invade other tissues and evade therapies. Phenotypic heterogeneity is the variety of functional properties and expressions of various lineage

*Corresponding author: Mansaru Seno, Department of Biotechnology and Drug Discovery, Graduate School of Interdisciplinary Science and Engineering in Health Systems, Okayama University, Okayama, Japan, E-mail: mansa@okayama-u.ac.jp

Citation: Seno M (2023) A Comparison of the Ability of Three Different Cancer Stem Cell Cultures to Spread. Int J Inflam Cancer Integr Ther, 10: 216.

Citation: Seno M (2023) A Comparison of the Ability of Three Different Cancer Stem Cell Cultures to Spread. Int J Inflam Cancer Integr Ther, 10: 216.

Page 2 of 3

markers that tumor cells can take on as the disease progresses. The intra- and inter-tumoral heterogeneity, which refers to the molecular differences brought about by tumor initiation in the same organ, can be used to classify tumors as well as biologically distinct disease entities. Cancer stem cells, or CSCs for short, are a distinct subpopulation of cells that are responsible for tumorigenesis. These cells are capable of self-renewal and differentiation, which helps to maintain the malignant growth and even encourage the invasion of adjacent tissues. CSCs are in charge of the new generation as well as those with differentiated phenotypes, whereas CSCs are in charge of the progeny and cancer-associated cells in the tumor. The question of where the CSCs came from remains unanswered [5].

The CSC niche, which CSCs can create, contains variant cellular components like cell-mediated adhesion and soluble factors, as well as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and tumor-associated neutrophils. The cells that make up the CSC niche play important roles in supporting CSC survival, such as encouraging angiogenesis, which leads to invasion and metastasis.

For the primary culture of metastatic tumors, intraperitoneal injection tumors and metastatic nodules from mice were separately excised, cut into pieces about 1 mm3 in size, and washed three times in Hanks' balanced salt solution (HBSS). The pieces were suspended in 4 mL of dissociation buffer that contained 1 mM CaCl2 in PBS, 0.25 percent trypsin, 0.1 percent collagenase, 20% KnockoutTM Serum Replacement, and 37 °C for one hour. To conclude the digestion, 5 milliliters of DMEM containing 10% FBS were added. For three minutes, the cellular suspension was centrifuged at 300 g. Then, at that point, the supernatant was moved to another 15-ml tube then, at that point, axis at 1000×g for 10 min. Without LIF, the cell pellet was suspended in adequate quantities of miPS medium. At a density of 3 105 cells per dish, the cells were seeded into a 60-mm dish coated with 1% gelatin. To get rid of the host cells, the cells were then given 1 g/mL of puromycin for a week. Using a light fluorescence Olympus IX81 microscope (Olympus, Tokyo, Japan), the expression of GFP and the morphology of the cells were observed and photographed.

Animal experiments

Charles River (Kanagawa, Japan) provided the immunodeficient nude mice (Balb/c-nu/nu, female, 4 weeks). After that, three mice from each cell line were injected with 1 106 cells suspended in sterile 200 l of HBSS intraperitoneally (i.p.). All tumors were resected and sectioned after six weeks for histologic analysis. Under the OKU-2020651, the ethics committee for animal experiments at Okayama University reviewed and approved each and every experiment on animals.

Histological analysis:

Immunohistochemistry

The procedure for immunohistochemistry (IHC) was as described previously. In a nutshell, 5 mm tissue sections were deparaffinized in xylene and gradually reduced in ethanol concentration to rehydrate them. The antigen epitopes were then unmasked using a standard microwave heating method in sodium citrate buffer (pH 6.0). Hydrogen peroxide and normal serum were used to block endogenous peroxidase and nonspecific epitopes, respectively [6]. The segments were brooded for the time being at 4 °C with the accompanying essential antibodies against GFP (#2956, Cell Flagging, Mama, USA), E-Cadherin (#3195, Cell Flagging, Mama, USA), and N-cadherin. After that, sections were incubated with the ABC reagent and biotinylated secondary antibodies from Vector Laboratories (Vector Laboratories, CA, USA). Horseradish

peroxidase (HRP) response was achieved with 3.3 -diaminobenzidine (Touch) substrates (Vector Research centers, CA, USA). Hematoxylin was used to counterstain the sections before they were mounted on a Micro mount [7].

RNA extraction and RT-qPCR

Following the manufacturer's instructions, total RNA was extracted using TRIzol RNA isolation reagents. To get rid of any remaining genomic DNA, the RNA was treated with DNase I. The optical density at 280 and 260 nm was measured with a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, DE, USA) to determine the concentration and purity of the RNA. Using a GoScriptTM Reverse Transcription System Kit, complementary DNA was prepared using 4 g of RNA in accordance with the manufacturer's instructions. RT-qPCR was carried out with a Light Cycler 480 SYBR green I Master Mix from Roche Diagnostics GmbH in Mannheim, Germany, in accordance with the directions provided by the manufacturer [8]. The gene of reference was GAPDH. The primer3 plus software and the blast primer tool were used to design and verify the specificity of primer sets.

RNA-sequencing and bioinformatic analysis

Segregation of all out RNA was proceeded as referenced previously. RNA tests were ready for sequencing utilizing NEBNext Ultra II RNA Library Prep Pack (New Britain Biolabs, Mama, USA). Novaseq6000 (Illumina, CA, USA) was used for the sequencing. The bioinformatic examination were completed with System stage < usegalaxy.org > utilizing Cuffdiff pipeline and the intensity map was created with coordinated Differential Articulation and Pathway investigation (iDEP).

Metastatic potential of cancer stem cell models

Using the conditioned medium (CM) of human breast cancer cell line BT549 cells, liver cancer cell line Huh7 cells, and mouse Lewis lung carcinoma (LLC) cell derived extracellular vesicles (EVs), our lab has previously established cancer stem cell (CSC) models, miPS-BT549cm [10], miPS-Huh7cm [8] and miPS-LLCev cells. 1a). Injection testing was used to first assess the tumorigenicity of these models. We used the cells that were isolated from the primary culture of each tumor for this study. Tumor-derived miPS-BT549cm and miPS-LLCev cells were injected subcutaneously. The tumor's miPS-Huh7cm cells were injected into the liver [9].

The metastasis was seen in cells in the liver, lung, and pancreas by the transplantation of miPS-BT549cm. The presence of metastasis was totally unique in relation to those got from miPS-LLCev cells. Unlike the nodules that were observed with miPS-LLCev cells, the tumors that developed in the liver were larger. In pancreas, the knobs were excessively little to be seen. Despite the fact that they were more modest than those on account of miPS-LLCev cells, the essential culture showed the presence of cells got from miPS-BT549cm cells by the statement of GFP. Spleen nodules and adhesions between the pancreas and spleen were unremarkable [10]. MiPS-BT549cm-Li-M, miPS-Bt549cm-Lu-M, miPS-BT549cm-Pa-M, and miPS-BT549cm-St-M cells were the primary cultured cells that were isolated from stomach metastasis, lung metastasis, pancreas metastasis, and liver metastasis, respectively.

Phenotypes of metastasized tumors were affected by the microenvironments

The metastasis of miPS-LLCev cells was spread in various organs like lung, liver, pancreas, and spleen . To begin, lung nodules that had formed within the alveolar inner layer revealed a significant

Citation: Seno M (2023) A Comparison of the Ability of Three Different Cancer Stem Cell Cultures to Spread. Int J Inflam Cancer Integr Ther, 10: 216.

Page 3 of 3

malignancy. Macroscopically, these metastatic nodules were large. The dangerous cells with pleomorphic cores, mitotic figures, necrotic regions, and angiogenesis were seen by hematoxylin and eosin stains. In addition, the knobs saw in liver were saturating between the hepatocytes without disturbance of the ordinary engineering of liver. Kupffer cells, blood sinusoids, and cirrhosis were the characteristics of the hepatic nodules. The atomic vacuolation in hepatocytes of knobs were unfilled or heterogeneous encompassed by basophilic film with unusual chromatin [11]. This perception is a movement step from cirrhosis to hepatocellular carcinoma. Additionally, the pancreas and spleen were the sites of growth for the metastatic tumors. Adenocarcinoma with obvious glandular structures, scattered mitogenic figures, adipocytes, and hemorrhage within the tumor tissues was found in sections of these tumors.

Discussion

An important example of metastasis models is the intraperitoneal and orthotopic transplantation of CSCs. These models help us understand the entire metastatic cascade, from the initial invasion to metastatic spread through intravasation, and they also help us develop new therapies for specific metastasis stages [12]. In this context, the intraperitoneal injection of CSCs demonstrated in this paper should provide a method for evaluating the metastatic potential of CSCs, which were isolated from primary tumors and prepared from miPSCs treated with various CM mimicking the "cancer-inducing niche." To evaluate the metastatic behaviors and differences among the CSCs, the metastatic abilities of three distinct CSC models made from the same source of miPS cells were also compared to one another.

The relative review showed that CSC models changed over from iPS cells ought to be an incredible asset in metastatic examinations. The CSCs, which came from the same type of iPS cell, had different metastatic potentials and preferred different organs to be released [13]. The examinations of the metastases and their atomic aggregates call attention to various degrees of cadherins and stemness markers in metastases of the three sorts of CSCs. We discovered that miPS-Huh7cm cells had distinctive metastasis in the intestines, whereas the CSC model created with LLC cells' extracellular vesicles had a high propensity to spread to the lung and grow into aggressive nodules [14]. Each portal seemed to be surrounded by nodules on the intestine. In addition, these characteristics were linked to elevated expression levels of metastatic driver genes like Twist, fibronectin, and vimentin in miPS-LLCev cells in comparison to the other two types of CSCs.

Conclusion

In conclusion, we demonstrate the establishment of a metastasis model using CSCs that have been transformed from iPS cells with success. The model will give researchers new tools for studying the process by which tumors start and spread. As a key factor regulating these characteristics, the microenvironment in which CSCs are generated may differ between CSCs in terms of their aggressiveness and metastatic ability.

Acknowledgement

Conflict of interest

None

References

1. Plaks , Kong N, Werb Z (2015) The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell. 16: 225-238.

2. Afify SM, Seno M (2019) Conversion of stem cells to cancer stem cells: undercurrent of cancer initiation. Cancers 11: 345.

3. Afify SM, Calle AS, Hassan G, Kumon K, Nawara HM, et al. (2020) A novel model of liver cancer stem cells developed from induced pluripotent stem cells. Br J Cancer 122: 1378-1390.

4. Osman A, Afify SM, Hassan G, Fu X, Seno A, et al. (2020) Revisiting cancer stem cells as the origin of cancer-associated cells in the tumor microenvironment: a hypothetical view from the potential of iPSCs. Cancers 12: 879.

5. Nair N, Calle AS, Zahra MH, Prieto-Vila M, Oo AKK, et al. (2017A cancer stem cell model as the point of origin of cancer-associated fibroblasts in tumor microenvironment. Sci Rep 7: 1-13.

6. Matsuda S, Yan T, Mizutani A, Sota T, Hiramoto Y, et al. (2014) Cancer stem cells maintain a hierarchy of differentiation by creating their niche. Int J Cancer 135: 27-36.

7. Prieto-Vila M, Yan T, Calle AS, Nair N, Hurley L, et al. (2016) iPSC-derived cancer stem cells provide a model of tumor vasculature. Am J Cancer Res 6: 1906.

8. Yan T, Mizutani A, Chen L, Takaki M, Hiramoto Y, et al. (2014) Characterization of cancer stem-like cells derived from mouse induced pluripotent stem cells transformed by tumor-derived extracellular vesicles. J Cancer 5: 572.

9. Chaffer CL, Thompson EW, Williams ED (2007) Mesenchymal to epithelial transition in development and disease. Cells Tissues Organs 185: 7-19.

10. Brabletz T, Jung A, Reu S, Porzner M, Hlubek F, et al. (2001) Variable β-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. Proc Natl Acad Sci USA 98: 10356-10361.

11. Valastyan S, Weinberg RA (2011) Tumor metastasis: molecular insights and evolving paradigms. Cell 147: 275-292.

12. Kessenbrock K, Plaks V, Werb Z (2010) Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141: 52-67.

13. Lambert AW, Pattabiraman DR, Weinberg RA (2017) Emerging biological principles of metastasis. Cell 168: 670-691.

14. Hugo H, Ackland ML, Blick T, Lawrence MG, Clements JA, et al. (2007) Epithelial—mesenchymal and mesenchymal—epithelial transitions in carcinoma progression. J Cell Physiol 213: 374-383.