

Stemness of breast cancer cells incubated on viscoelastic gel substrates

Abstract

Acrylamide copolymer-based gel substrates with different viscoelasticity were employed to evaluate the viscoelasticity effect on the direct relation among cancer stemness and mesenchymal properties with induction of epithelial–mesenchymal transition (EMT) of human breast adenocarcinoma (MCF-7) cells in both normoxia and hypoxia. The softer gel substrate produced a large amount of surface molecule of cancer stem cells (CSC) marker CD44. In contrast, for the stem cell biomarker CD133 expression, their coefficient of damping ($\tan\delta$)-dependent manner was not contributed by EMT phenomenon and was an independent from acquisition of the EMT. The substrate damping as potential physical parameter emerged the important linkage to cancer stemness and EMT induction.

Keywords: viscoelastic gel substrates, cancer cells, epithelial–mesenchymal transition, gene expression, cancer stem cells

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Abbreviations: CSC, cancer stem cells; EMT, epithelial–mesenchymal transition; DMEM, dulbecco’s modified eagle’s medium

Introduction

Cancer ranks as the leading causes of mortality in Japan.¹ A recently proposed hypothesis suggests that cancer stem cells (CSCs) and epithelial–mesenchymal transition (EMT) play a pivotal role in cancer metastasis, recurrence and drug resistance.² A few CSCs do self-renew and produce a large number of heterogeneous and highly proliferative cancer cells to form primary tumors. Thus, CSCs survive and cause cancer recurrence. Recent studies showed that inducible factors of EMT not only induce EMT, but also enhance CSC features of cancer cells.² These findings suggest that EMT is closely related to cancer progression.

Our previous study showed that scaffold-induced EMT of mammary carcinoma cells could be established in vitro by using a polymer cell culture scaffold with a three-dimensional fibrous porous structure.³ In addition, the relationship between EMT and the motility of mammary carcinoma cells under both hypoxia and normoxia was studied using polymeric gel substrates with different viscoelasticity, which mimic in vivo micro-environment.⁴

In this study, we aimed at examining the effect of viscoelasticity of the substrate on the direct relation among cancer stemness and mesenchymal properties with induction of EMT in both normoxia and hypoxia.

Experimental

Cell culture: Human breast adenocarcinoma cell line, MCF-7 (ATCC) were cultured in high glucose Dulbecco’s modified Eagle’s medium (DMEM) (Nacalai Tesque, Japan) supplemented with 10% (v/v) FBS, grown at 37°C under 5% CO₂ atmosphere and 95% relative humidity (normoxia) or hypoxic condition (94% N₂, 5% CO₂, and 1% O₂) at 37°C.

CSC marker expression

MCF-7 cells were seeded at a density of 5.0×10^3 cells/cm² on eight different substrates, and cultured for 7 days. The substrates were copolymerized gels (P(AAm-co-ACA)) (AC)⁴ made from copolymers of

Acrylamide (AAm) and N-acryloyl-6-aminocaproic acid (ACA) made into six different hardness, and coated with Cellmatrix Type I-C (Nitta Gelatin). In addition cell culture plates (TCP: control) and collagen-coat coated with TCP and Cellmatrix Type I-C were used. After culturing, the expression levels of CD133 and CD44 were measured by fluorescence excitation preparative assay using a flow cytometer (Attune NXT Acoustic Focusing Cytometer, Thermo Fisher Sci. Flow Jo (ver 10) was used as analysis software.

Gene expression analysis

The expression level of HIF-1 α , TGF- β , vimentin, CDH2, CDH1, SNAI2, ZEB1 were evaluated by RT-PCR.⁴ Statistical analysis was performed using Tukey–Kramer method.

Results and discussion

CSC marker expression

The expression rates of CD44 and CD133 in AC gels as a function of viscoelasticity are shown in Figure 1. The CD133+ levels increased the loss factor ($\tan\delta$) of AC gels to around 0.09 under both oxygen conditions and then decreased. The CD44+ levels increased with the increase in $\tan\delta$ under both O₂ conditions, indicating for the first time that the expression of CD133 and CD44 is not related to each other under different O₂ conditions in MCF-7 cells. CD44 is known to bind mainly to hyaluronan and is involved in the maintenance of CSCs. On the other hand, the expression of CD133 has been found to correlate with poor prognosis.⁵ Since each transmembrane protein contributes to different functions, it is suggested that the dependence on $\tan\delta$ is also different.

Gene expression

MCF-7 cells cultured in AC gels with $\tan\delta = 0.244$ (AC-soft) and $\tan\delta = 0.195$ (AC-mid) showed significant changes in vimentin expression under both oxygen concentration conditions. The expression of vimentin under hypoxia was significantly increased by 720-fold in AC-soft and 360-fold in AC-mid gels compared to AC gels with $\tan\delta = 0.0437$ (AC-stiff). The expression of CDH2 was similar to that of vimentin, and the expression of CDH2 was 440-fold higher in AC-soft than in AC-stiff under hypoxia.

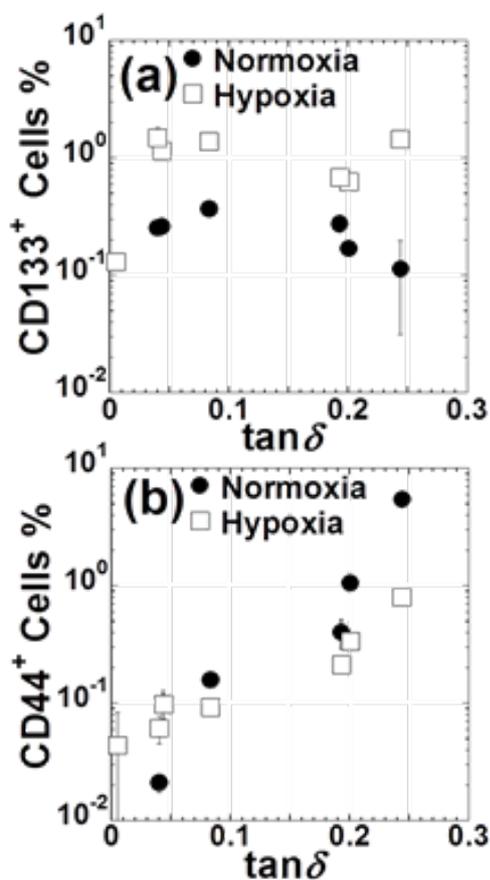


Figure 1 Flow cytometry analysis of (a) CD133, (b) CD44, expression level of MCF-7 cells incubated on different viscoelastic gel substrates and collagen-coated TCP under both oxygen concentration conditions for 7 days. Data were expressed as mean \pm SD ($n=3$).

Conclusion

When MCF-7 cells were cultured on AC-soft, EMT was sufficiently enhanced under both oxygenation conditions. In the cells cultured on

AC-soft substrate, there was a significant association between CD44 expression and vimentin/CDH2 expression, indicating that cells on soft AC gel scaffold were favored for survival by EMT. On the other hand, the $\tan \delta$ -dependence of CD133 expression was not related to the acquisition of EMT. $\tan \delta$ was shown to be a physical parameter with important relevance for MCF-7 cell stemness and EMT induction. The effectiveness of environmental stimulus properties as a feature of $\tan \delta$ was found for the first time.

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Author Contributions

Contributed to the initiating idea and performed most of the experiments: Ohta Y, Sasaki R, and Okamoto M. Supervised research: Okamoto M. The manuscript was written through contributions of all authors.

Conflicts of interest

Author declares that there is no conflicts of interest.

References

1. Health, Labour and Welfare Ministry, Japan. 2020.
2. Craene BD, Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer*. 2013;13:97–110.
3. Domura R, Sasaki R, Okamoto M, et al. Comprehensive study on cellular morphologies, proliferation, motility, and epithelial–mesenchymal transition of breast cancer cells incubated on electrospun polymeric fiber substrates. *J Mater Chem B*. 2017;5:2588–2600.
4. Ishikawa Y, Sasaki R, Domura R, et al. Cellular morphologies, motility, and epithelial–mesenchymal transition of breast cancer cells incubated on viscoelastic gel substrates in hypoxia. *Mater Today Chem*. 2019;13:8–17.
5. Chen S, Song X, Chen Z, et al. CD133 expression and the prognosis of colorectal cancer: a systematic review and meta-analysis. *PLoS One*. 2013;8:e56380.