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研究課題名（和文）ステロイドホルモンのがん幹細胞への作用を介した放射線に対する乳腺応答の修飾

研究課題名（英文）Modulation of breast radiation responses by steroid hormones through its action on cancer stem cells

研究代表者

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研究成果の概要（和文）：プロゲステロン(Pg)受容体を持たない基底様細胞であるMCF10AではPg＋放射線照射でがんや幹細胞性の関連するmiRNA制御が生じ、放射線抵抗性のCSCを増加させる。Pgは膜型Pg受容体を介して惹起した。PI3/Akt経路の阻害はPg＋放射線照射によるCSCの生成を抑制した。膜型Pg受容体を経たPI3/Akt経路の惹起はFOXO転写活性の不活性化、snailやslug発現の増加、miR-29の発現抑制、そして乳がんCSCの維持に必要な転写因子、KLF4の増加につながる。miR-29発現の安定化ががん細胞の生成を妨げていて、その抑制だけで十分なCSC生成を引き起こした。

研究成果の概要（英文）：Progesterone (Pg) stimulated cancer stem cell expansion both in progesterone receptor (PR)-positive breast cancer cells and in PR-negative normal cells. In MCF10A basal-like PR-negative cells, Pg treatment and irradiation triggered cancer and stemness-associated microRNA regulations (such as the downregulation of miR-22 and miR-29c), which resulted in increased proportions of radiation-resistant CSCs. Pg activated the PI3k/Akt pathway via membrane progesterone receptor (mPR). Inhibition of the PI3k/Akt pathway counteracted the generation of CSCs by Pg and irradiation. The stimulation of PI3K/Akt via mPR resulted in the inactivation of FOXO transcriptional activity, the upregulation of snail and slug expression and a downregulation of miR-29 expression, which led to increased levels of KLF4, a transcription factor required for breast CSC maintenance. Stabilization of miR-29 expression impeded the generation of CSCs, while its inhibition alone was sufficient to generate CSCs.

研究分野：　Cancer Research, Radiation Research

キーワード：　cancer stem cell　breast cancer　radiation　progesterone　steroid hormone　microRNA

１．研究開始当初の背景

Breast cancer is responsible for 13.7% of all cancer deaths worldwide. Although much progress has been made in the understanding and cure of breast cancer, significant challenges remain. Among the four main subtypes of breast cancer, basal-like breast cancer (BBC) is of particular interest, due to its high frequency, relative lack of effective therapies and poor prognosis. BBC represents the predominant subtype of triple-negative (TN) breast cancer Ionizing radiation (for example as treatment for other cancers) and cumulative exposure to steroid hormones (as seen in postmenopausal women under hormone replacement therapy) are known risk factors for breast cancer. Although basal mammary cells do not express hormonal receptors (progesterone and estrogen receptors) and TN breast cancer does not respond to hormonal therapy, a new family of G-protein coupled membrane progestin receptors (mPRs) has been identified as in intermediary of progesterone signaling PR– cell lines, as well as in normal and breast cancer tissue, including TN/BBC. mPRs initiate various signaling pathways associated with G-protein activation. There is now wide acknowledgment that tumors are generally heterogeneous and that cancer treatment failure, relapse or metastasis may be related a small population of stem-like cells that are capable of self-renewing and of causing the different lineage of cancer cells comprising a tumor. These cancer stem cells (CSCs, also referred to as “tumor-initiating cells” or stem-like cells) are more radio-resistant than their non-CSC counterparts and have a distinct molecular signature. Furthermore, CSCs and differentiated cancer cells seem to coexist in a dynamic equilibrium influenced by the micro-environment and by specific micro-RNA expression patterns. The development of new therapeutic strategies that selectively target CSCs is currently receiving increasing attention from the academic research community and the pharmaceutical industry. A better understanding of the initiation and maintenance mechanisms of CSCs is therefore necessary, in order to ultimately identify potential molecular targets.

２．研究の目的

Exposure to ionizing radiation was shown to result in an increased risk of breast cancer. There is strong evidence that steroid hormones (progesterone, estrogens) influence radiation sensitivity and breast cancer risk.
We first wanted to assess whether the modulation of radiation-induced breast cancer risk by steroid hormones could involve cancer stem cells (CSCs). In agreement with this hypothesis, we could observe the generation of radioresistant breast CSCs by ionizing radiation and exposure to progesterone. Surprisingly, MCF10A cells were also responsive to progesterone treatment, although they did not express progesterone receptor (PR). Therefore, we then investigated molecular pathways involved in the initiation and maintenance of basal-like CSCs via membrane progestin receptors (mPR).

３．研究の方法

MCF10A breast cell line and two breast cancer cell lines (MCF7 and T-47D) were cultured as monolayer. CSCs were identified and sorted by flow cytometry based on the expression of specific cell surface markers (CD44+/CD24-) or intrinsic activity (ALDH+).
FOXO reporter assay was performed using a PI3K/Akt pathway-responsive FOXO luciferase reporter vector.
For mammosphere culture, sorted cells were seeded in ultra-low adherent plates and spheres were counted after 7 days.
Real-time PCR measurement of snail, slug, Klf4, mPRs, nuclear PR gene expression levels was also performed. Cells were treated with progesterone, Caffeic acid phenetyhyl ester (CAPE, a NFkB inhibitor), wortmannin (a PI3K inhibitor), Org-OD-02-0 (a mPR agonist), mifepristone (RU-486), a PR antagonist.

４．研究成果

First, we observed that progesterone stimulated the expansion of the radioresistant CSC compartment both in nuclear progesterone receptor (PR)-positive breast cancer cells and in PR-negative normal cells. In MCF10A normal epithelial basal-like PR-negative cells, progesterone treatment and X-ray radiation exposure triggered cancer and stemness-associated microRNA regulations (such as the downregulation of miR-22 and miR-29c expression) (Figure 1), which resulted in increased proportions of radiation-resistant tumor-initiating ALDH+ and CD44+/CD24- CSCs (Vares et al., 2013). The combined effects of irradiation and progesterone on tumor-initiating CSCs might thus result in additional cancer risk. In the normal breast, PR is expressed only in a subset of luminal epithelial cells. The existence of PR-independent effects need to be taken into account.

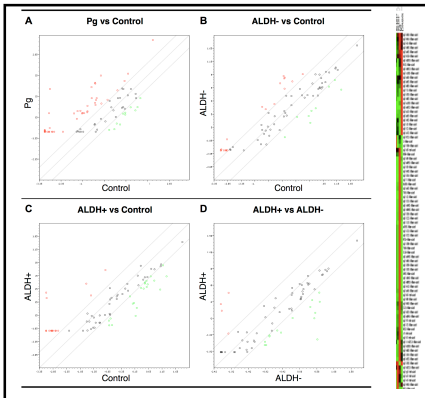

Figure 1: Stemness and cancer-associated miRNA regulations after progesterone treatment and irradiation in MCF10A cells.

We hypothesized that progesterone effects in MCF10A cells rely on membrane progestin receptors (mPRs). We verified the expression of several mPR isoforms in those cells.
We then discovered that progesterone activated the PI3k/Akt pathway via mPR. Inhibition of the PI3k/Akt pathway counteracted the generation of CSCs by progesterone and irradiation. The stimulation of PI3K/Akt via mPR resulted in the inactivation of FOXO transcriptional activity, the upregulation of snail and slug expression and a downregulation of miR-29 expression, which led to increased levels of KLF4, a transcription factor required for breast CSC maintenance (Figure 2). Stabilization of miR-29 expression impeded the generation of CSCs, while its inhibition alone was sufficient to generate CSCs (Vares et al., 2015) (Figure 3).

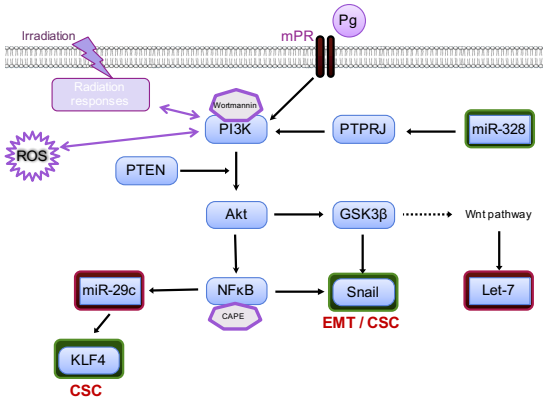

Figure 2: Basal-like cancer stem cell initiation model.

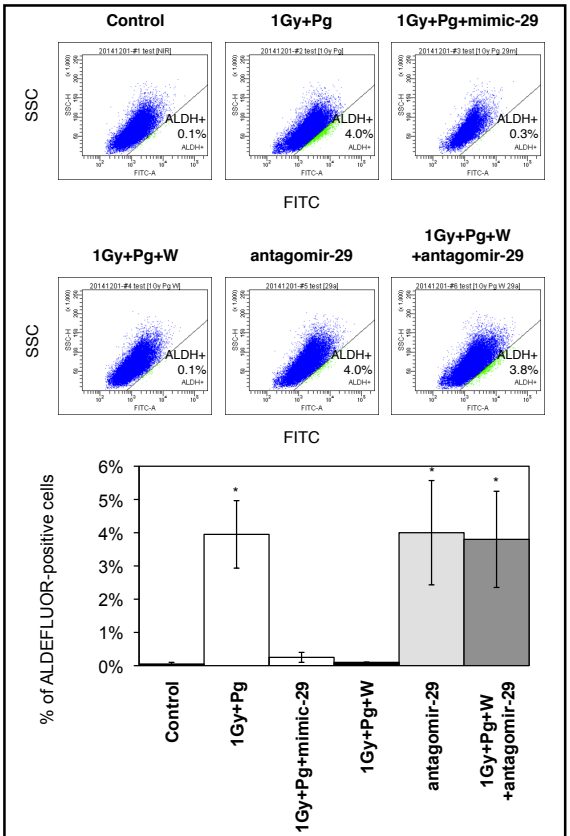

Figure 3: The modulation of miR-29 activity influences the proportion of ALDH+ CSCs.

This suggests that miR-29 plays a critical in CSC initiation and maintenance in this model, and maybe in other models. Indeed, aberrant expression of tumor-suppressor miR-29

family is observed in many cancers. These findings have important implications for understanding potential cancer risk resulting from the stimulation of basal cells. They also provide new insights into the role of progesterone in regulating radiation responses and cancer risk.
Furthermore, the identification of CSC-associated micro-RNA regulations may allow us to establishing new anti-CSC strategies.Therapeutic modulation of micro-RNA activity is an emerging area of research which has demonstrated promising potential and is well tolerated *in vivo*. Coupled with innovative radiation therapy modalities, this strategy should lead to new cancer treatment modalities and new CSC-targeting drugs.
We would now like to expand these findings and describe mechanisms responsible for CSC initiation and maintenance in basal-like breast cancer and other challenging cancer models. Based on these new data, we will devise potential anti-CSC strategies targeting signaling pathways and miRNAs associated with CSC initiation, maintenance and resistance to radiation/chemotherapy.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　　件）
Vares G, Sai S, Wang B, Fujimori A, Nenoi M, Nakajima T. Progesterone generates cancer stem cells through membrane progesterone receptor-triggered signaling in basal-like human mammary cells. Cancer Lett. 2015 Jul 1;362(2):167-73. doi: 10.1016/j.canlet.2015.03.030.

Vares G, Cui X, Wang B, Nakajima T, Nenoi M. Generation of breast cancer stem cells by steroid hormones in irradiated human mammary cell lines. PLoS One. 2013 Oct 16;8(10):e77124. doi:10.1371/journal.pone.0077124

〔学会発表〕（計　　件）
International conferences :*

(* I also attended domestic conferences, such as the annual Japan Cancer Association Annual Meeting)

- G. Vares, S. Sai, B. Wang, A. Fujimori, M. Nenoi, T. Nakajima.　Progesterone generates cancer stem cells through membrane progesterone receptor-triggered signaling in basal-like human mammary cells. International Congress for Radiation Research (ICRR). Kyoto, Japan, 2015.05.25-29.

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〔図書〕（計　　件）

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番号：
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〔その他〕
ホームページ等

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