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# 科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Stromal Actions in TNBC

研究課題名（英文）Stromal Actions in TNBC

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研究成果の概要（和文）：腫瘍微小環境は多くの癌において、癌を進行させる重要な役割を担っている。本研究では癌におけるアンドロゲン合成が間質由来の因子によりどのような影響を受けるかを明らかにすることを目的とした。トリプルネガティブ乳癌（TNBC）においてCAFsは代表的なアンドロゲン合成酵素の発現と活性を上昇させることを明らかにした。パラクリン作用によりアンドロゲン合成に影響を与える可能性のある因子としてIL-6とHGFを選択し、ヒト乳癌初代培養CAFsとTNBC培養細胞を用いた細胞培養実験によりこれらの因子がアンドロゲン合成に影響を与えることを示した。

研究成果の概要（英文）：The tumor microenviroment plays pivitol roles in the initiation and promotion of many malignancies. The goal of this project was to determine if androgen metabolism in the tumor may be impacted by stromal factors. We demonstrated that CAFs increased the expression and activities of major androgen creating enzymes (17BHSD2, 17BHSD5 and 5aR1) in TNBC. To understand the underlying mechanisms cytokine array analysis was used to screen for secreted cytokines from primary human CAFs and these were then cross referenced with cytokines known to impact androgen metabolism. IL-6 and HGF were selected as potential paracrine mediators and their ability to affect androgen metabolism was demonstrated. To generalise from the reductionist context of cell culture CAF markers were examined in a TNBC cohort and found to be significantly correlated with 17BHSD2 and 17BHSD5 expression in TNBC tissues, especially in AR positive cases. This braodly suggests CAF regulation of androgen metabolism in TNBC.

研究分野：　Breast Cancer

キーワード：　Breast Cancer　TNBC　Stroma　Androgens

## １．研究開始当初の背景

Triple negative breast cancer is characterized by lack of ER, PR and Her2. As these proteins are essential components of the pathways that breast cancer targeted therapy is directed at, their absence in this particular type of breast cancer makes it particularly difficult to treat. This characteristic possibly contributes to its current adverse prognosis. In addition to poor prognosis, TNBC cancers are characterized by the heterogeneity of the tumor types encompassed in this group and the heterogeneity and genomic instability observed within individual tumors. While many studies, including our own, have focused on the expression of target proteins in carcinoma cells, comparatively little attention has been paid to the role of the stroma, and potential target proteins in the stroma in TNBC. This is especially surprising given the growing understanding of the importance of metabolic and immune alterations in cancer adjacent stroma both in breast carcinomas and other tumor types.

One of the most promising targets is the androgen receptor (AR) due to its expression in up to 30% of TNBC patients and the availability of existing drugs that target AR. We and others have previously shown AR expression is associated with less aggressive variants of TNBC. Despite this, clinical trials of androgen antagonists in ER- and mostly TNBC metastatic patients have begun and preliminary data suggests a positive response in a subset of patents after 6 months. While apparently contradictory the current consensus is that while AR is associated with better prognosis as it suggests a less aggressive variant, in a subset of TNBC it can drive cancer development, hence the therapeutic response observed in a subset of AR antagonist treated patients. This is analogous to the more commonly accepted situation with the ERα; despite its presence indicating a less aggressive cancer, ERα is still a driver and thus a good therapeutic target. However, what however so far has been omitted in the consideration of AR as a therapeutic target in TNBC is potential actions of AR in stromal cells. It is know that AR is present in both NAFs and CAFs from ER positive breast cancers (Knower 2013, Breast Cancer Res Treat 142 (1):211-223), interacts with some of the carcinoma and stromal processes. However the role of the stroma in reacting to or directing androgen metabolism in not well known.

## ２．研究の目的

The goal of this research was to determine how stromal interactions impact androgen signaling pathways in triple negative breast cancers.

## ３．研究の方法

To answer the question above this research employed two main modes.

Firstly we utilized immunohistologial investigations in large case series of triple negative breast cancer patients. Our total cohort was made up of 110 Japanese patients diagnosed with operable invasive triple negative breast cancers. The goal of these studies was to determine how transformation of fibroblasts into CAFs may affect androgen metabolism in the tissue and what effects this may mean in terms of long term prognosis. This allows us to understand our findings at a population level.

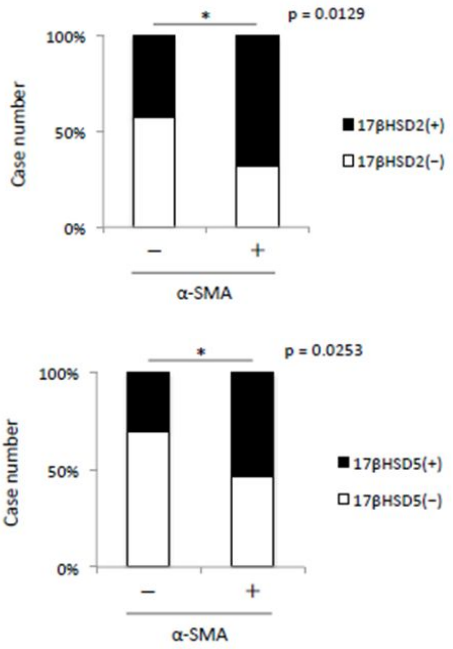
The second mode of investigation was the use of co-culture of triple negative epithelial cell lines with primary (directly derived from tumors) human stromal fibroblast. These experiments allowed us understand interaction between stromal pathways and androgen signaling at a mechanistic level. These interactions were then interrogated at multiple levels including; functional analysis of androgenic enzyme activity by GC-MSMS, Screening of cytokine secretions through a cytokine array, Molecular analysis through real time PCR and western blotting, and functional analysis of cell behavior by proliferation and invasion assays.

## ４．研究成果

**(1) CAF marker expression was confirmed in TNBC as was associated with androgenic enzymes.**

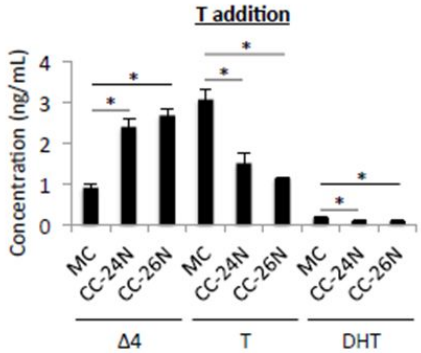
We used the standard definitions of loss of CD34 and acquisition of alpha-smooth muscle actin to define CAFs in triple negative breast cancer tissues. The presence of CAFs in pathological samples strongly correlated with the presence of androgenic enzyme sin the carcinoma cells

suggesting CAFs may play some role in regulating androgen metabolism.

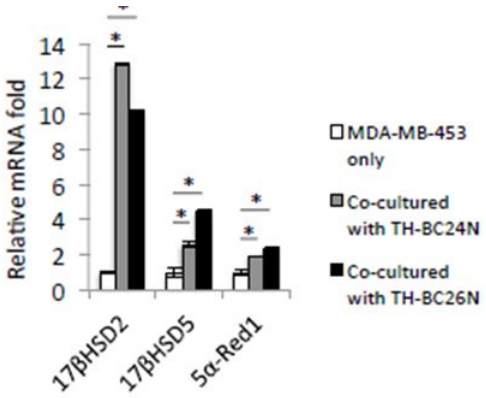



**(2) Co-culture of TNBC cell lines with CAFs results in changes in androgen metabolism.**

In order to determine if there was effect of CAFs on androgen production we co-culture of TNBC cell lines with primary stromal derived fibroblast that fit the CAF criteria above. We then took the cell culture media from these samples and analysed the levels of steroids, which is directly indicative of the function of steroid enzymes. In this analysis we observed that co-culture with CAFs increased androgen levels in the media suggesting an effect of CAFs on the levels of conversion of androgens.

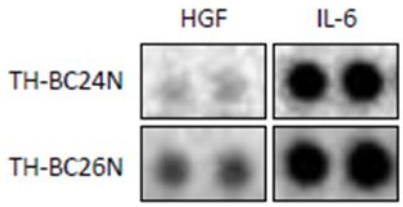



We confirmed that these changes in the metabolic products of the enzymes was due to changes in the expression levels of the enzymes in the epithelial cells

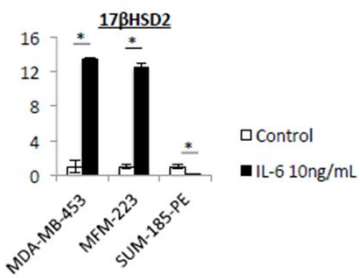


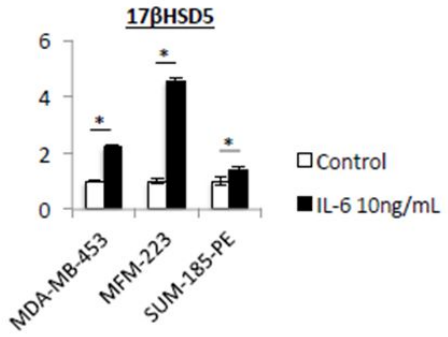

**(3) CAFs release a wide range of cytokines, some of which are associated with the regulation of androgenic enzymes**

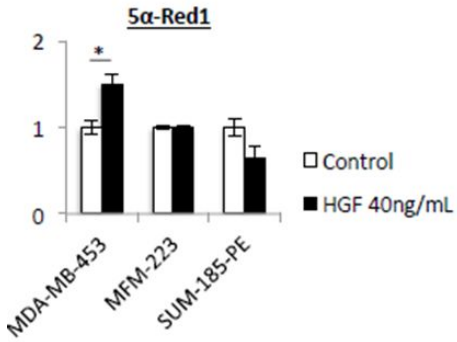
Through the use of a cytokine array we were able to screen the two primary CAFs available to us in order to determine their cytokine profile. We then cross referenced the highly expressed cytokines with those that have been shown in other malignancies to regulate androgen metabolism. From this we focused on Il-6 and HGF as potential regulators of androgen metabolism as they were expressed at high levels by both CAF cell lines and have been previously suggested as androgen metabolism regulators.




Direct treatment of triple negative cell lines with these factors led to alterations in the expression of steroidogenic enzymes,






This suggests that influence through paracrine factors may be one way by which stromal cells can influence the androgen metabolism in epithelial cells and has importance relevance for the development of androgen directed therapies in breast cancers.

**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）**

**〔学会発表〕（計　　件）**

**〔図書〕（計　　件）**

**〔産業財産権〕**

**○出願状況（計　　件）**

**名称：**
**発明者：**
**権利者：**
**種類：**
**番号：**
**出願年月日：**
**国内外の別：**

**○取得状況（計　　件）**

**名称：**
**発明者：**
**権利者：**
**種類：**
**番号：**
**取得年月日：**
**国内外の別：**

**〔その他〕**
**ホームページ等**

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