Inhibiting C-Jun to Retard Cell Proliferation Promoted by AP-2β in the Breast Cancer

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Abstract: AP-2β is a molecular marker in breast cancer cells without too much attention before. A tight association between AP-2β overexpression and breast cancer was reported. This study investigates whether c-jun is the certain down-stream protein of AP-2β in regulating the cell proliferation in breast cancer. The expression of AP-2β in human cell line will be measure by Western Blot. Tumor size *in vivo* will be measured by volume. The possible results include: the tumor growth of positive control is slower than negative control group, the tumor is increasingly faster growing compared with negative control, or the tumor is growing in the same speed in both negative and positive control groups. The result will indicate whether C-jun is a key down-stream target for mediating the tumor-promoting role of AP-2β.

1 INTRODUCTION

The breast cancer is one of the most threatening type of cancers for women worldwide. The most significant four subtypes are: Luminal A or HR+/HER2- (HR-positive/HER2-negative), Luminal B or HR+/HER2+ (HR-positive/HER2-positive), Triple-negative or HR-/HER2- (HR/HER2-negative) and HER2-positive. (American Cancer Society. 2019)

There are many additional molecular factors included in developing breast cancer. Among them, the activator protein-2 family of transcription factor is very common. They have five members: AP-2α, -2β, -2γ, -2δ, -2ε. They are encoded by separate genes (TFAP2A, TFAP2B, TFAP2C, TFAP2D, TFAP2E). They are thought to bond with specific DNA sequence as an activator or repressor to stimulate or terminate the growth process of cells. (Turner et al 1998) Regarding breast cancer, most studies focus on AP-2α and AP-2γ. The expression of AP-2β in breast cancer has only started to be noticed and studied in recent years.

The tight association of AP-2β with breast cancer has been observed. (Raap et al 2018) AP-2β is a new mammary epithelial differentiation marker and its over expression leads to cell proliferation in breast cancer. With knockdown of AP-2β , the cell proliferation is downregulated. But at the same time, in the previous experiment, proteins included in developing the breast cancer also get down-regulated expression such as p75, MMP-2, MMP-9, C-Jun, p-ERK and STAT3. The expression levels of p75, MMP-2, MMP-9, C-Jun, p-ERK and STAT3 show obvious upregulation following overexpression of AP-2β. (Li, Xu, Luo, Hao, Zhao, Yu et al 2018) To investigate more about AP-2β underlying mechanism in developing breast cancer, I hypothesize the c-jun is a key downstream protein of AP-2β in regulating cell proliferation. The possible signaling pathway involving C-Jun is shown in Figure 1. AP-2β is the transcription factor that contacts the promoter or enhancer to regulate transcription. C-Jun is supposed to be a member of downstream pathway in the hypothesis. The complete downstream pathway is still not fully understood. AP-2β may be regulate the process via MEK/ERK/c-jun pathway indicated by previous study, or maybe through PI3K/Akt pathway which is also indicated in previous study, or actually via various pathways together (See Figure 2). (MAKOTO, Toru, Masaki, Koichi 2011) The receptors that are responsible for transducing signals are still under investigation. The estrogen-receptor is already proven to be independent from expression of AP-2β. (Raap et al 2018), However, other receptors included in breast cancer, such as progesterone receptors, HER2 receptors and EGFR receptors are still being studied to confirm whether they are associated with the expression of AP-2β.

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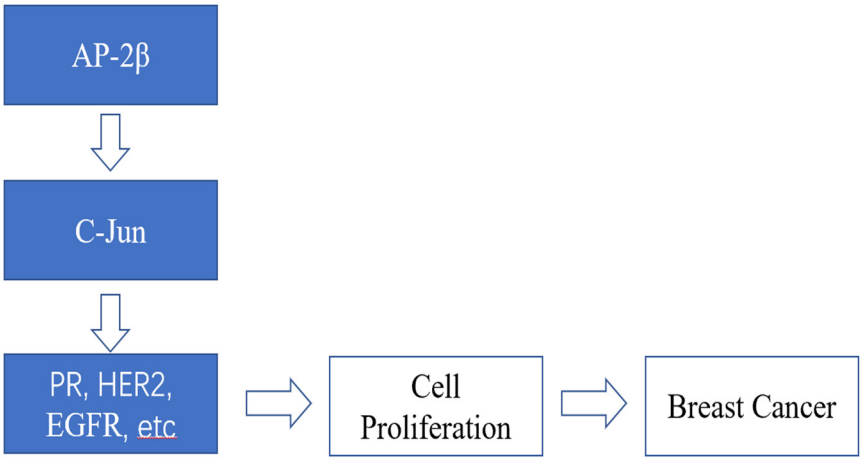

Figure 1: C-Jun Pathway. This figure shows the position and activities of C-Jun in the AP-2β oncogenic pathway.

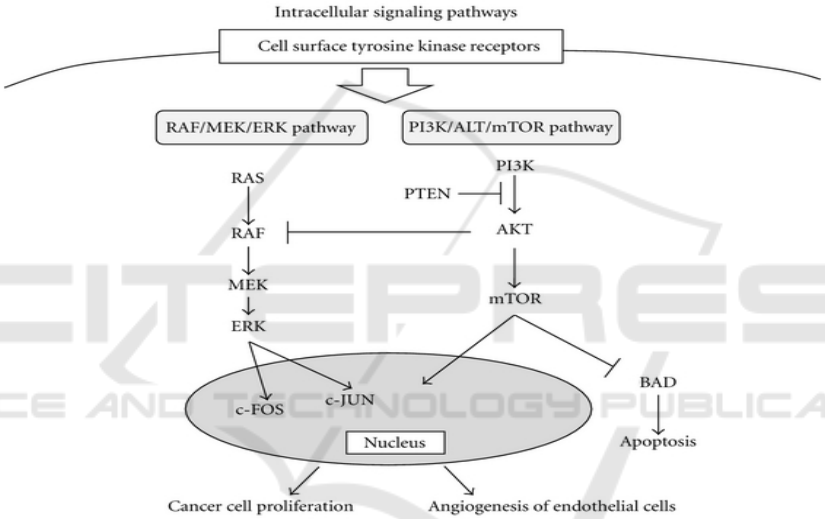

Figure 2: RAF/MEK/ERK signalling pathway is shown. Growth factors promoting cell proliferation activate the RAF/MEK/ERK pathway. MEK regulates the intermediate signalling by phosphorylation and activation of the downstream ERK molecule. ERK regulates cellular activity, indirect inducers of gene expression and transcription factors of the AP-1 family, such as c-JUN and c-FOS. (MAKOTO, Toru, Masaki, Koichi 2011)

2 METHODS

2.1 Materials

Human breast cancer cell line MDA-MB-231
Xenograft mouse models
C-jun inhibitor JNK-JN-8 (Ebelt ND et al 2017)

2.2 In vitro Cell Culture

The cell line is cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 10 µg/ml bovine insulin, 2.5 g/l glucose, 1 mM sodium pyruvate, 2 mM glutamine, and 10 mM HEPES, in a water-saturated atmosphere containing 5% CO2 at 37.5°C. (Raap et al 2018) MDA-MB-231 cells will be divided into two groups: negative control and positive control. In the negative control group, the cells will be injected with saline. In the positive control group, the cells will be injected with c-jun inhibitor (JNK-IN-8). The results will be analyzed by observing the cell proliferation.

2.3 Flow Cytometry

The cell proliferation will be measured through flow cytometry system by ThermoFisher Scientific. The

cell line sample will be measured every 2 days after injecting JUN-JN-8.

2.4 AP-2β Western Blot

Total cellular proteins are separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. Membranes are probed with anti-AP-2β (H-87, 1:1000, Santa Cruz Biotechnology) (Raap et al 2018).

2.5 Animal Model

The xenograft mice will be divided into two groups of 4: negative control and positive control. For the mice in the negative control group, the mice will be injected with saline. For the positive control group, MDA-MB-231 cells will be injected subcutaneously into the left flank of each mouse in the same volume as the saline injected in the mice in negative control group. The tumor growth will be monitored every four days after the tenth day of injecting MDA-MB-231 cells by measuring the tumor size. The tumor's size will be measured in the terms of volume and the calculation equation is V= (width × length) /2. The tumors will be weighed after death. (Li, Xu, Luo, Hao, Zhao, Yu et al 2018)

2.6 Statistics

The western blot and immunohistochemistry will be repeated three times for each group. To compare data obtained in the positive and negative control groups, a student t test will be displayed and $P \leq 0.05$ is considered significant. (Li, Xu, Luo, Hao, Zhao, Yu et al 2018)

3 RESULTS (OVERVIEW SHOWN IN TABLE 1)

3.1 Possible Results 1: Applying JUN-JN-8 Inhibits the Breast Cancer Cell Proliferation in MDA-MB-231 Cell Line and the Tumor Cells in Mouse Model

Inhibiting c-jun leads to significantly slower cell proliferation in the breast cancer cells and the stop or much slower growth of the tumor in the mice of positive control group.

3.2 Possible Result 2: Applying JUN-JN-8 Inhibits the Breast Cancer Cell Proliferation in MDA-MB-231 Cell Line, But Not the Tumor Cells in Mouse Model

Inhibiting c-jun leads to no effect on tumor growth although the cell proliferation is reduced in the *In Vitro* cell culture experiment.

3.3 Possible Result 3: Applying JUN-JN-8 Inhibits the Breast Cancer Cell Proliferation in MDA-MB-231 Cell Line, but Promote the Breast Cancer Cell Proliferation in Mouse Model

Inhibiting c-jun leads to confusingly much quicker enlargement of the tumor in the condition that the cell proliferation is inhibited in the breast cancer cells of *In Vitro* conditions.

3.4 Possible Result 4: Applying JUN-JN-8 Inhibits the Breast Cancer Cell Proliferation in Mouse Model, But Not the MDA-MB-231 Cell Line

Injecting c-jun inhibitor (JNK-IN-8) do not show inhibiting effect on breast cancer cell proliferation in the cell line. However, when MDA-MB-231 cells that are injected with JNK-IN-8 are injected into the mouse, the tumor growth speed is slowing down.

3.5 Possible Result 5: Applying JUN-JN-K Does Not Inhibit the Breast Cancer Cell Proliferation in Both MDA-MB-231 Cell Line and the Mouse Model

Injecting Both In vitro and In vivo experiments show that injecting c-jun inhibitor has no effect on inhibiting the breast cancer cell proliferation and tumor growth.

Table 1: Possible Results.

Cell lines	Result 1	Result 2	Result 3	Result 4	Result 5	Result 6
In vivo Model	+	-	?	+	-	?
MDA-MB-231	+	+	+	-	-	-
Note. "+" represents a a significant decrease in cell proliferation/tumor growth speed of mouse. "-" represents not significantly different from negative control.						

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3.6 Possible Result 6: Applying JUN-JN-8 Does Not Inhibit the Breast Cancer Cell Proliferation in MDA-MB-231 and Promote the Breast Cancer Cell Proliferation in Mouse Model

In the condition of no difference shown in the inhibiting cell proliferation compared with negative control group in the MDA-MB-231, the mouse shows quicker tumor growth.

4 DISCUSSION

Previous studies have found that silencing AP-2β leads to downregulation of the expression of a number of proteins, including c-jun. Possible downstream pathways are MEK-ERK-c-jun and MEK/STAT3/MMPs. It is therefore reasonable to assume that c-jun may be a direct downstream protein of AP-2β or one of these direct proteins.

Possible Result 1 is consistent with previous studies that downregulation of AP-2β led to a downregulation of c-jun protein levels. Previous studies have shown that the amount of c-jun protein is influenced by AP-2β. C-jun regulation by AP-2β most likely promotes tumor cell proliferation through the MEK/ERK/c-jun signalling pathway, indicated by previous experiment.

Possible Result 2 contradicts the hypothesis. C-jun is inhibited by JUN-JN-8 in *in vitro* human breast cancer cell line and cell proliferation is also inhibited, suggesting that c-jun is one of the potential direct downstream proteins. However, tumor cell proliferation is not inhibited in the mouse model, suggesting that there are other signalling pathways at work in the mouse that disable the inhibition of c-jun and thus do not inhibit the regulation of AP-2B, or that there are other AP-2B downstream proteins at work in the mouse that counteract the proliferative effects of c-jun inhibition.

Possible Result 3 contradicts the hypothesis. The inhibition of the cell proliferation process can only suggest that c-jun can play a role in mediating cell proliferation, but cannot be characterized as a direct downstream protein of AP-2β. As there are other potential direct downstream proteins that mediate the tumor-promoting effects of AP-2β, the underlying mechanisms are unclear. In previous studies, when AP-2β was downregulated, the number of many other proteins was also reduced, such as MMP9, MMP2 and p75 shown in Figure 3. (Li, Xu, Luo, Hao, Zhao, Yu et al 2018)

Possible Result 4 are contradictory to the hypothesis, as they do not provide clear evidence to identify c-jun as a direct downstream target of AP-2β. Tumor growth in mice is shown to be inhibited when no inhibition is shown on MDA-MB-231. This may demonstrate that c-jun has no direct role in mediating the cell proliferation pathway, but that it can signal to other downstream proteins that promote the tumor pathway of AP-2β.

Possible Result 5 strongly suggests that c-jun is not a direct downstream protein of AP-2β. and that it has no therapeutic effect on breast cancer.

Possible Result 6 shows an unlikely result, but it is also possible that it will emerge in future experiments. Cell proliferation will not be inhibited when c-jun inhibitors are injected into breast cancer cell lines. This is not evidence to determine whether c-jun is a direct downstream protein of AP-2β, which may have multiple signalling pathways. However, in mice injected with c-jun inhibitor-treated MDA-MB-231, tumor growth will become more pronounced, a possible outcome that is very strange and if it occurs more research is needed to explore the mechanisms involved.

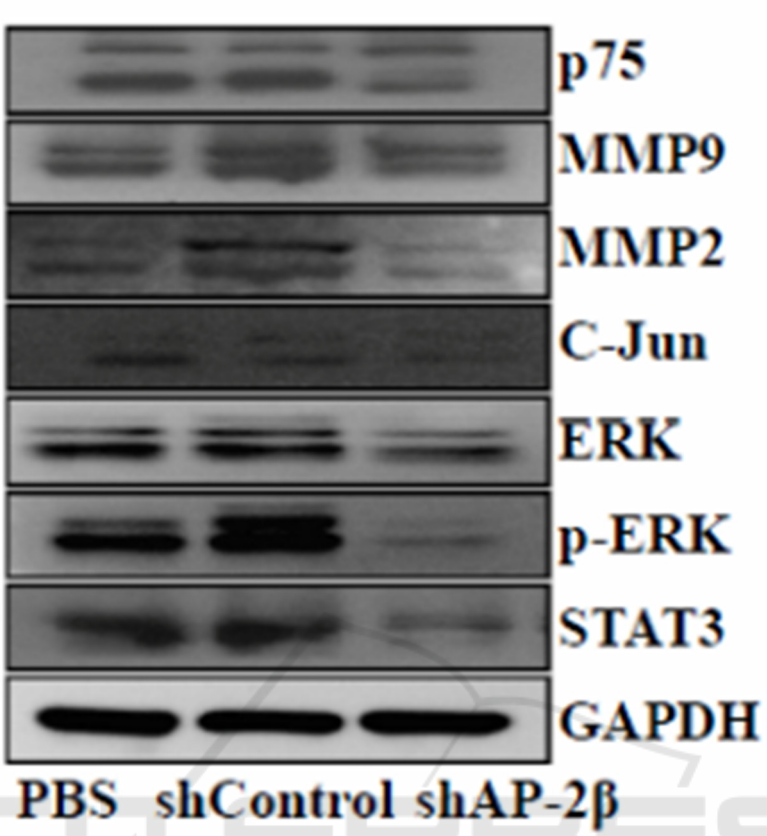

Figure 3: Expression levels of P75, MMP2, MMP9, C-Jun, STAT3 measured by Western blot in MDA-MB-231 cells following AP-2β knockdown. (Li, Xu, Luo, Hao, Zhao, Yu et al 2018)

5 CONCLUSIONS

C-jun may be an important protein in the downstream pathway of AP-2β during tumour growth in breast cancer as experiments have shown that c-jun expression is closely associated with AP-2β. Overall, this article discusses the downstream pathway component of AP-2β in the mechanisms underlying the promotion of breast cancer cell proliferation and uses in vitro cell lines and mouse models to determine whether c-jun is one of the important downstream proteins. Our findings will indicate whether c-jun could be a potential target for blocking breast cancer tumour growth. Results that contradict the hypothesis would also indicate the existence of other downstream protein proteins regulated by AP-2β, in both respects a confirmation of potential therapeutic targets in breast cancer in the future. As no previous attention has been paid to the role of AP-2β in breast cancer, research into its complete mechanism in breast cancer progression is limited. More research on how AP-2β regulates cell proliferation still needs to be clearly detailed in order to find more effective targets for the treatment of breast cancer.

REFERENCES

American Cancer Society. (2019) Breast Cancer Facts & Figures 2019-2020. Atlanta: American Cancer Society, Inc. 2019. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/breast-cancer-facts-and-figures/breast-cancer-facts-and-figures-2019-2020.pdf

Ebelt ND, Kaoud TS, Edupuganti R, Van Ravenstein S, Dalby KN, Van Den Berg CL. A c-Jun N-terminal kinase inhibitor, JNK-IN-8, sensitizes triple negative breast cancer cells to lapatinib. Oncotarget. 2017;8(62):104894.

Li Z, Xu X, Luo M, Hao J, Zhao S, Yu W, et al. Activator protein-2β promotes tumor growth and predicts poor prognosis in breast cancer. Cellular Physiology and Biochemistry. 2018;47(5):1925-35.

MAKOTO M, Toru M, Masaki K, Koichi H. The Molecular Pathogenesis and Clinical Implications of

Hepatocellular Carcinoma. International Journal of Hepatology 2011(2):818672.

Raap M, Gronewold M, Christgen H, Glage S, Bentires-Alj M, Koren S, et al. Lobular carcinoma in situ and invasive lobular breast cancer are characterized by enhanced expression of transcription factor AP-2beta. Lab Invest. 2018;98(1):117-29.

Turner BC, Zhang J, Gumbs AA, Maher MG, Kaplan L, Carter D, et al. Expression of AP-2 transcription factors in human breast cancer correlates with the regulation of multiple growth factor signalling pathways. Cancer research. 1998;58(23):5466-72.