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ANTI-CSC COMBINATION THERAPY USING WNT SIGNALING-TARGETED DRUG

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ABSTRACT

Dysfunctions of Wnt, Hedgehog and Notch pathways are evident in multiple tumor types and malignancies. A number of studies have suggested that dysregulation of Wnt/β-catenin signaling occurs in human breast cancer. Specifically, inhibition of Wnt/ β-catenin pathway is implicated in arresting of cancer stem cells (CSCs), a small subset of cancer cells capable of self-renewal and differentiation into heterogeneous tumor cells. Here, we investigated tumor initiating property of breast cancer stem cell *in-vitro* with XAV-939 an inhibitor of Wnt/β-catenin signaling pathway. Targeting Wnt/β-catenin signaling with this inhibitor represents a promising strategy to suppress metastasis.

Keywords: Cancer Stem Cell, 3D Mammosphere, Wnt/β-catenin, metastasis, CD44+/CD24

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INTRODUCTION

Amongst women, breast cancer is the most common cancer in both developed and developing countries of the globe, and the second reason of mortality in developed countries after lung cancer [1]. Breast cancer death rate has been reducing in most high-income regions of North America and Europe due to screening, early detection and admission to adjuvant treatments [2, 3]. Screening programs are assessed to have declined breast cancer mortality in 20% in the invited population [4, 5]. Moreover, breast carcinoma is one of the fields in cancer that has perceived some major advances in the understanding of the fundamental biology with the molecular characterization into discrete entities, as well as changes in terms of treatment and prognosis in the last fifteen years, as demonstrated by the use of trastuzumab for the adjuvant treatment of women with HER-2 overexpressing early-stage and advanced breast cancer [6]. Whereas these developments have improved patient outcomes, some disease continue to be resistant to current treatments are still poorly understood. Also, both incidence and mortality from breast cancer are increasing in moderate-income countries of the world where significant challenges remain.

The conventional treatments like chemotherapy and radiotherapy fails to cure the disease most of the time and this may be due to the presence of cancer stem cells (CSCs). Cancer metastasis, resistance to therapies and disease recurrence are significant hurdles to successful treatment of breast cancer. Cancer stem cells (CSCs) are a small subset of cancer cells with the capability of self-renewal and differentiation into heterogeneous tumor cells, and they have been believed to be responsible for tumor initiation, growth, and recurrence [7]. First, CSCs possess a high tumor-initiating capacity, which is an essential characteristic that enables the formation of new tumours [8]. Moreover, CSCs also associated an epithelial-mesenchymal transition (EMT) marker

which helps the tumor cells to migrate into other organs and tissues [9].

Wnt signaling is essential for normal breast stem cell function and mammary gland development. Numbers of studies have shown that aberrant Wnt signaling in breast cancer stem cells is a crucial event in breast tumorigenesis [10]. Abnormal activation of Wnt signaling has been implicated in the regulation of a plethora of CSC types including colorectal cancer, breast cancer, hematologic cancer, skin cancer, liver cancer and lung cancer [11, 12, 13, 14, 15, 16]. Recent study also demonstrated that Wnt/β-catenin signaling activity is higher in breast CSCs than the bulk tumor population [17]. Markers like $CD44^{+}/CD24^{-}$ have been proposed to exhibit enhanced tumorigenic and metastatic properties in tumor xenograft models [18,19].

Here, we investigated the effects of inhibiting Wnt/β-catenin signaling on breast cancer stem cell to link its tumor initiating ability through 3D mammosphere formation. Targeting Wnt/β-catenin signaling with this XAV-939 inhibitor represents a promising strategy to suppress metastasis.

MATERIALS & METHODS

Cell lines and Culture Conditions

TMD-231 breast carcinoma cell line was obtained directly from the ATCC (Manassas, VA, USA), and grown in DMEM (Thermo Fisher Scientific, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO) at 37°C in a 5% humidified CO_2incubator. Characterization of cell line was done according to their surface expression phenotype.TMD-231 cell lines were grown for 48 hours with 80% confluency. After that the same cell lines were treated with XAV-939 inhibitor for 72 hours. These cells were used to form 3D mammosphere along with controls.XAV-939 Inhibitor was purchased from selleckchem (USA).

3D Mammosphere formation

Single cell suspensions were plated in 6-well tissue culture plates covered with poly-2-hydroxyethyl-methacrylate (Sigma, St. Louis, MO) to prevent cell attachment, at a density of 1,000 cells/ml in serum-free DMEM supplemented with 1% L-glutamine, 1% penicillin/streptomycin, 30% F12 (Sigma), 2% B27 (Thermo Fisher Scientific, Carlsbad, CA), 20 ng/ml EGF (Sigma, St. Louis, MO) and 20 ng/ml FGFb (Thermo Fisher Scientific, Carlsbad, CA). The medium was made semi-solid by the addition of 0.5% Methylcellulose (R&D Systems, Minneapolis, MN) to prevent cell aggregation. After 7 days in culture, mammospheres were collected by gentle centrifugation (200 x g) and dissociated enzymatically (5 min in 1:1 trypsin/DMEM solution at 37°C) and mechanically by passing through a 25G needle (6 strokes). Single cells were re-plated at a density of 1,000 cells/ml for subsequent passages.

Viability Assay

TMD-231 cells were grown in a 96-well tissue culture plate with drugs and appropriate controls. Subsequently they were incubated with the WST-1 reagent (Dojindo Inc, Japan) for 4 hours. After this incubation period, the formazan dye formed was quantitated with a multi-well spectrophotometer (ELISA reader). The measured absorbance directly correlates to the number of viable cells.

Flow cytometry

PE-conjugated CD44, FITC-conjugated CD24 monoclonal antibody was purchased from BD Bioscience. After 3 days of drug treatment the TMD-231 cells were dissociated with 0.25 % trypsin-EDTA (1 mM) (Invitrogen) for 3 min and washed with Calcium and magnesium free dulbecco phosphate buffered saline solution by spinning at 400g for 7 minutes. Then these cells were diluted in 100 µl FACS buffer (PBS containing 1 % fetal calf serum) and then incubated for 1 hr at 4 °C in FACS buffer with the corresponding mAb: anti-CD44-PE, CD24-FITC. Flow cytometry analysis was performed with a BD FACSCanto II flow cytometer (BD Biosciences).

RESULTS

Viability of TMD-231-Cell lines in the presence of XAV-939 inhibitor

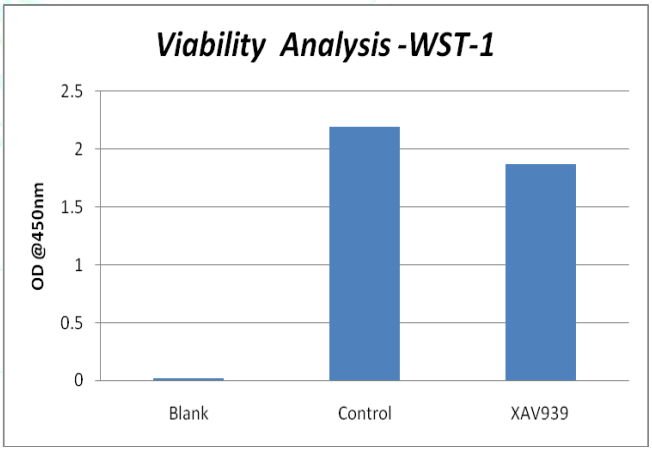
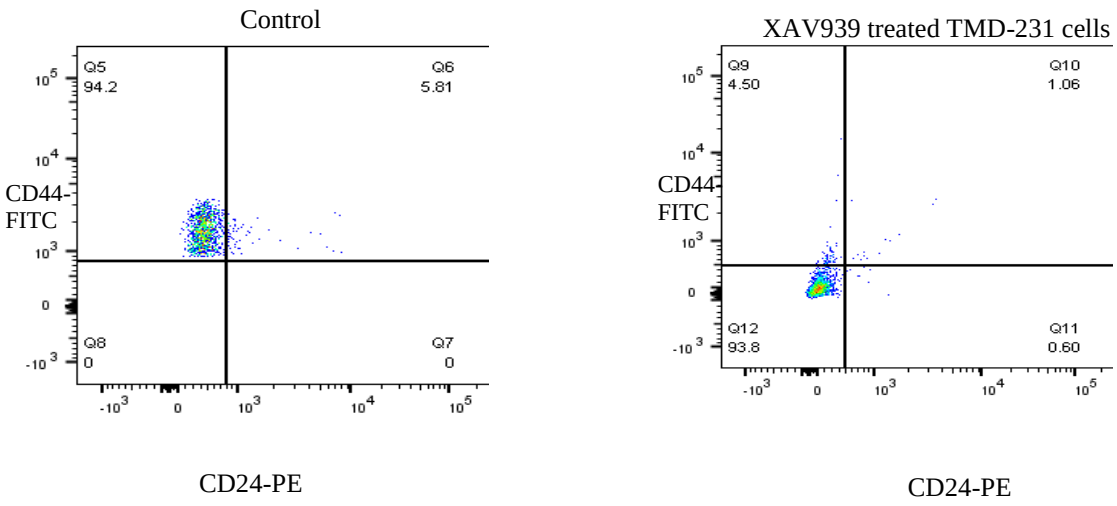


Figure 1: The graph representing the viability of control and XAV-939 treated TMD-231 cell lines at day 3 culture. The absorbance readings were taken at 450 nm.

After 3 days of XAV-939 treatment, TMD-231 cell lines were measured for the viability by WST-1 method. We observed very minimal or negligible difference between control and XAV-939 treated cell lines, and the same was observed in the morphological images under inverted microscope (data not shown here). This result indicates, even though XAV-939 play a major role in suppressing wnt β-catenin signaling pathway it did not affect the viability of the cells.



Expression profiles of breast CSC markers

Figure 2: Flow cytometry analysis representing the expression of breast cancer stem cell markers CD44+/CD24-. A] In control, the expression of CD44/CD24 was 6.05%. B] In XAV-939 treated cells the expression of CD44 was 1.04%.

We examined the expression profiles of breast cancer stem cell markers in TMD-231 cells with or without Wnt1 inhibitor i.e. XAV-939. We noticed significant decrease of breast cancer stem cell markers $CD44^{+}/CD24^{-}$ when compared to the control. However, in control the expression pattern of breast cancer stem cell was unchanged. These findings were very evident that wnt/β-catenin signaling plays a vital role in cancer stem cell. So, arresting or blocking the wnt-βcatenin signaling can suppress the breast cancer stem cell which involves in multiple roles like tumorigenesis, chemoresistance and tumors relapse.

Suppression of metastasis

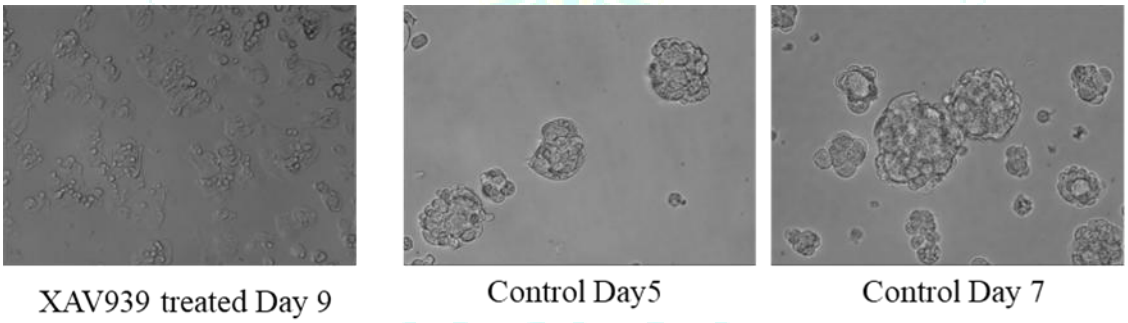


Figure 3: 3D Mammosphere formation assay. The first image is pre XAV-939 treated cells, no mammosphere was observed even after 9 days of spheroid culture. Second image, TMD-231 cells forming 3D mammosphere at day 5. Third image, TMD-231 cells forming mammosphere at day 7.

To further confirm the effects of Wnt/β-catenin signaling on tumor cells we formed 3D mammospehres. We used XAV-939 treated cells along with normal TMD-231 cells as a control for spheroid formation assay. Interestingly the data was very much correlated with the flow cytometry analysis. The spheroids were started forming at day 3 itself (data not shown here) in control whereas in XAV-939 treated cells we couldn't find any mammosphere even after 9^{th} day of culture. These results suggest that wnt-β catenin signaling is very much required for tumor initiating capability and metastasis which are conferred by CSCs.

DISCUSSION

Like in any other cancers, breast cancer also fails to respond to their current chemotherapies. The resistance and recurrence are contributed by cancer stem cells. Hence, the identification of CSCs in breast cancer represents an important milestone in the understanding of chemo-drug resistance and cancer recurrence. Targeting and eradication of these cells represents a potential strategy to improve the clinical outcomes.

Recent understandings of the biological characteristics of breast cancer stem cells have facilitated the identification of mechanisms underlying the development of malignant breast cancer. One such mechanism is dysregulation of Wnt/β-catenin signaling occurs in breast cancer [20]. In this context, abnormal Wnt/β-catenin signaling activity may be an important clinical and pathologic feature of breast cancer and a predictor of poor overall survival [21]. XAV-939 specifically inhibits tankyrase PARP activity.

XAV-939 deregulates the Wnt/b-catenin pathway which has been implicated in many cancers including breast. Moreover, tankyrase 1 (TNKS1) inhibition may in part blocked Wnt/β-catenin signaling and reduced the expression of anti-apoptosis protein. Also, inhibition of TNKS1 reduced colony formation in vitro [22].

In this study, we treated TMD-231 cell lines with XAV-939, an inhibitor of Wnt/β-catenin signaling pathway that resulted in 1) decreased levels of the stem cell markers CD44+/CD24-2) Reduced 3D mammosphere formation. However, there were not much difference in their cell viability.

CONCLUSION

Tumor initiating ability is one of the important characteristics of cancer stem cell. In breast cancer presence of these cancer stem cells notably the $CD44^{+}/CD24^{-}$ subpopulation is enriched under suspension sphere culture conditions. Abolishing the 3D mammosphere forming capacity in the TMD-231 cancer cell line is a direct indication of suppression of metastasis with a blockade in Wnt/β-catenin signaling pathway.

REFERENCES

1. Ferlay J., Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015; 136(5):E359–E386.
2. DeSantis CE., Bray F., Ferlay J., et al. International variation in female breast cancer incidence and mortality rates. Cancer Epidemiol Biomarkers Prev, 2015; 24(10):1495–1506.
3. Hashim D., Boffetta P., La Vecchia C., et al. The global decrease in cancer mortality: trends and disparities. Ann Oncol, 2016; 27(5):926–933.
4. Independent UK Panel on Breast Cancer Screening. The benefits and harms of breast cancer screening: an independent review. Lancet, 2012; 380(9855): 1778–1786.
5. Pace LE., Keating NL. A systematic assessment of benefits and risks to guide breast cancer screening decisions. JAMA, 2014; 311(13):1327–1335.
6. Moja L., Tagliabue L., Balduzzi S., et al. Trastuzumab containing regimens for early breast cancer. Cochrane Database Syst Rev, 2012; 4:CD006243.
7. Koury J., Zhong L., Hao J. Targeting Signaling Pathways in Cancer Stem Cells for Cancer Treatment. Stem Cells International, 2017; 2017:2925869. doi:10.1155/2017/2925869.
8. Hermann P.C., et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell, 2007; 1:313–23.
9. Mani S.A., et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell, 2008; 133:704–15.
10. Howard B., Ashworth A. Signaling pathways implicated in early mammary gland morphogenesis and breast cancer. PLoS Genet, 2006; 2:e112.
11. Basu S., Haase G., Ben-Ze'ev A. Wnt signaling in cancer stem cells and colon cancer metastasis. F1000 Research, 2016; 5(699).
12. Mukherjee S., Mazumdar M., Chakraborty S., et al. Curcumin inhibits breast cancer stem cell migration by amplifying the E-cadherin/β-catenin negative feedback loop. Stem Cell Research and Therapy, 2014; 5 (5):116.
13. Reya T., Duncan A. W., Ailles L., et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature, 2003; 423(6938):409–414.
14. Zhang X., Lou Y., Wang H., et al. Wnt signaling regulates the stemness of lung cancer stem cells and its inhibitors exert anticancer effect on lung cancer SPC-A1 cells. Medical Oncology. 2015; 32(4): 95.
15. Vasanthakumar S., Sasikala P., Padma M., et al. EpCAM as a novel therapeutic target for hepatocellular carcinoma. Journal of Oncological Sciences, 2007; 3:71-76.
16. Malanchi I., Peinado H., Kassen D., et al. Cutaneous cancer stem cell maintenance is dependent on β-catenin signalling. Nature, 2008; 452(7187):650–653.
17. Jang GB., Kim JY., Cho SD., Park KS., Jung JY., Lee HY., et al. Blockade of Wnt/beta-catenin signaling suppresses breast cancer metastasis by inhibiting CSC-like phenotype. Sci Rep, 2015; 5:12465.
18. Al-Hajj M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J. & Clarke, M.F. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci, 2003; USA100:3983–8.
19. Liu, H et al. Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. Proc Natl Acad Sci, 2010; USA107:18115–20.
20. Zardawi S.J., O'Toole S.A., Sutherland R.L. & Musgrove, E.A. Dysregulation of Hedgehog, Wnt and Notch signalling pathways in breast cancer. Histol Histopathol, 2009; 24:385–98.
21. Lopez-Knowles E.et al. Cytoplasmic localization of beta-catenin is a marker of poor outcome in breast cancer patients. Cancer Epidemiol Biomarkers Prev, 2010; 19:301–9.
22. Xiao-Hong T., Wei-Jian H. Yan F., et al. XAV939, a tankyrase 1 inhibitior, promotes cell apoptosis in neuroblastoma cell lines by inhibiting Wnt/β-catenin signaling pathway. J Exp & Clin Cancer Res, 2013; 32:100.