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14. ABSTRACT

Cancer stem cells (CSCs) can initiate and sustain tumor growth and escape chemo/radiation therapies, result in cancer relapse and poor prognoses. Epithelial Mesenchymal transition (EMT) occurred in CSCs are responsible for cancer invasion and metastasis. SIRT1, a class III histone deacetylase was previously described to promote breast cancer stem cells (BCSCs), however, the association between SIRT1 and breast cancer is uncertain. In this study, we detected SIRT1 possessed high expressions in higher grade of breast cancer patients which harbor CSC properties, and SIRT1 expression is associated with cancer stem cells in breast cancer specimen by ALDH1a/CD44 double staining. SIRT1 inhibitors significantly reduced breast cancer stem cell population by flow cytometry study using CD24/CD44 and ALDH1a. SIRT1 inhibition greatly down-regulate the genes of cancer stem cells such as Nanog and SOX2, and genes of EMT markers such as vimentin. In xenograft study, SIRT1 inhibitor cambinol significantly inhibited tumor growth and completely blocked tumor cell metastasis comparing with the control mice. SIRT1 inhibitors also inhibit the drug resistance to cisplatin of tumor cells. Our results showed SIRT1 regulate cancer stem cells through Wnt/β-catenin pathway and DVL-3 appears to be important regulate factor. Our results suggest that SIRT1 potentially acts as a prognostic factor in breast cancer and plays an important role to promote BCSCs. Inhibition of SIRT1 may have significant therapeutic value in breast cancer.

15. SUBJECT TERMS

Breast cancer, cancer stem cell, SIRT1,SIRT1 inhibitor, exnograft model, EMT, Wnt, DVL3

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1. Introduction

Breast cancer is the most common malignant disease in women worldwide. The overall survival of breast cancer patients is greatly extended due to the improvement of diagnosis and treatment. However, there is a subpopulation of cancer cells, cancer stem cells (CSCs), which cannot be eliminated by current therapies, and resulted to recurrence and metastasis of breast cancer. Cancer stem cells (CSCs) are a rare population of cancer cell, they possess the characteristics of self-renewal and initiate and sustain tumor growth. Breast cancer stem cells (BCSCs) harbor $CD44^{high}/CD24^{low}$ and ALDEFLOUR-positive (ALDH1) properties, and some epithelial mesenchymal transition (EMT) markers. EMT represents the series of events converting adherent epithelial cells into individual migratory cells able to invade the extracellular matrix, and EMT plays a crucial role in cancer cell invasion and the distal metastasis in epithelial cancers. CSCs are largely regulated by Wnt/ β-catenin, Notch and Hedgehog pathways. These pathways are often dysregulated in many types of cancers, specifically within subpopulations of these cancers that possess stem cell properties. Therefore, it becomes important for understanding the features of cancer stem cells and blocking their activities in cancer therapy. Sirtuin 1 (SIRT1) belongs to a class III histone deacetylase (HDAC) that deacetylates histone and non-histone proteins to regulate gene transcription factors and protein functions. SIRT1 regulations are involved in cell growth, apoptosis and tumorigesis. Recently, SIRT1 was found to play essential roles in the maintenance and differentiation of various cancer stem cells, moreover, it was described SIRT1 has strong expressions in many malignant diseases included breast cancer. SIRT1 has described to involve in several signal pathways to regulation, such as Bcl-2 and Wnt/β-catenin pathway. Therefore, SIRT1 is considered as an important role in tumorigenesis and a close correlation with cancer stem cells. However, the role of how SIRT1 associates with breast cancer stem cells is unclear.

For clarify those issues, we investigated how SIRT1 regulates BCSCs, then we tested the therapeutic effects of SIRT1 inhibition in xenograft mice carrying breast tumors. Finally we elucidated the underlying mechanisms of SIRT1 regulation to BCSCs.

2. Keywords

Breast cancer, breast cancer stem cell (BCSC), epithelial mesenchymal transformation (EMT), invasion, migration, SIRT1, inhibitor, cambinol, EX527, Wnt pathway, xenograft, immunohistochemistry, near-infrared fluorescent (NIRF) dye, tissue growth factor, cancer cell line, lymphovascular invasion, flow cytometry, qRT-PCR

3. Overall Project Summary

We designed 6 tasks to finish this project.

Task 1. SIRT1 inhibitors can induce differentiation of CSCs in breast cancer cell lines.

Task 2. Human breast cancer cells (from patient's samples) with CSC features have SIRT1.

Task 3. SIRT1 inhibition can decrease metastasis, induce differentiation of CSCs, reduce EMT, and increase tumor cell sensitivity to chemotherapy in xenograft mouse model.

Task 4. Wnt pathway is highly activated in breast CSCs and EMT of human breast cancer specimens.

Task 5. Wnt pathway is blocked in the SIRT1 inhibition xenograft tumor tissue, which is responsible for inducing differentiation of CSCs and reducing EMT (merged to task 3 and 4).

Task 6. Using cell line *in vitro* study to demonstrate that SIRT1 regulates CSCs and EMT through activation of Wnt pathway via interaction with Dvl proteins.

All tasks have been completed and we are in the process for submitting the manuscripts for paper publications.

Task 1. SIRT1 inhibitors can induce differentiation of CSCs in breast cancer cell lines.

The task has been performed and completed. In order to study the breast cancer stem cell population in cancer cell lines, flow cytometry analysis for the most commonly used stem cell markers CD44/CD24 expression and ALDH1a activity had been used, and mammosphere functional assay also was performed.

Breast cancer cell lines (MDA-MB-231, MDA-MB-478 and T-47D) were treated with SIRT1 inhibitor cambinol and EX527. Flow cytometry analysis for CD44/CD24 expression showed a significant reduced expression of CD44 expression in the triple negative breast cancer cell line MDA-MB-231. MDA-MB-231 cancer cells were negative for CD24 and positive for CD44, supporting the high grade nature of the tumor cells. When the cells were treated with SIRT1 inhibitors cambinol (25 µM) and Ex527 (50 µM) for 24 hours, the cells showed significantly decreasing CD44 expression with a marked left shift (Figure 1A and 1B). Another triple negative breast cancer MDA-MB-468 cells showed coexpression of both CD24 and CD44, which was different from MDA-MB-231. Studies had shown that CD24 expression in MDA-MB-468 cells was important for its invasive ability. With SIRT1 inhibitors cambinol (25 µM) and Ex527 (50 µM) treatment, MDA-MB-468 cells showed significant loss of CD24 expression and only slight loss of CD44 (Figure 2A and 2B). The hormonal receptor positive, high grade breast cancer T-47D cells had no significant CD44 expression. All studies had been repeated for at least 2 times and showed similar results.

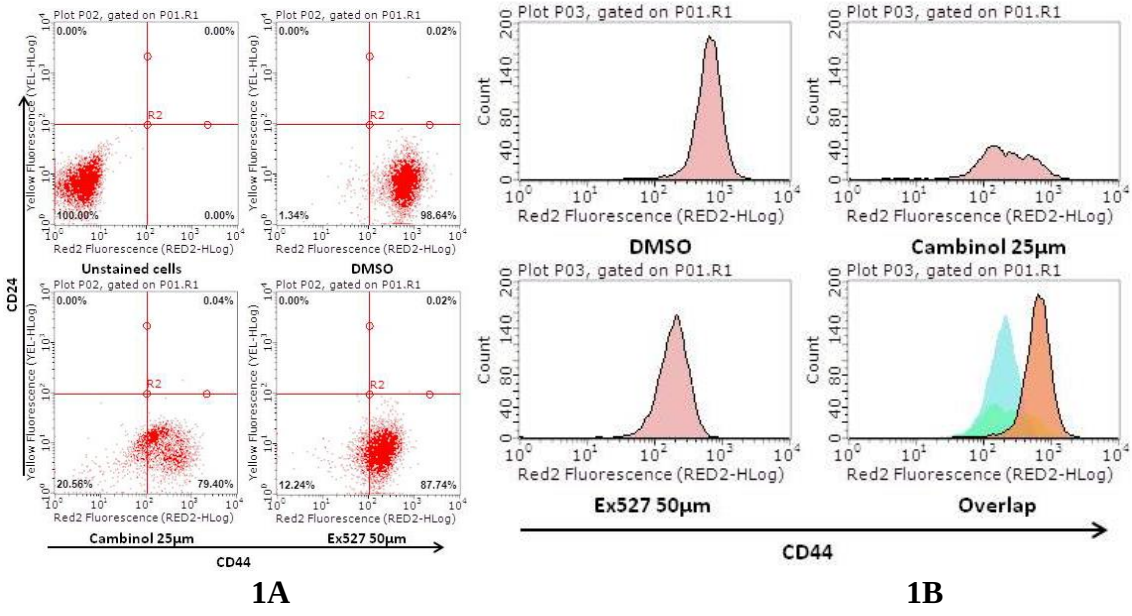


Figure 1. Figure 1A shows the dot plot of flow cytometry analysis for CD24/CD44 expression in MDA-MB-231 cancer cells, and 1B shows the histograph and the overlap of the CD44 intensity in SIRT1 inhibitor treated cells comparing to DMSO control (orange-control; green-cambinol treated; blue-Ex527 treated).

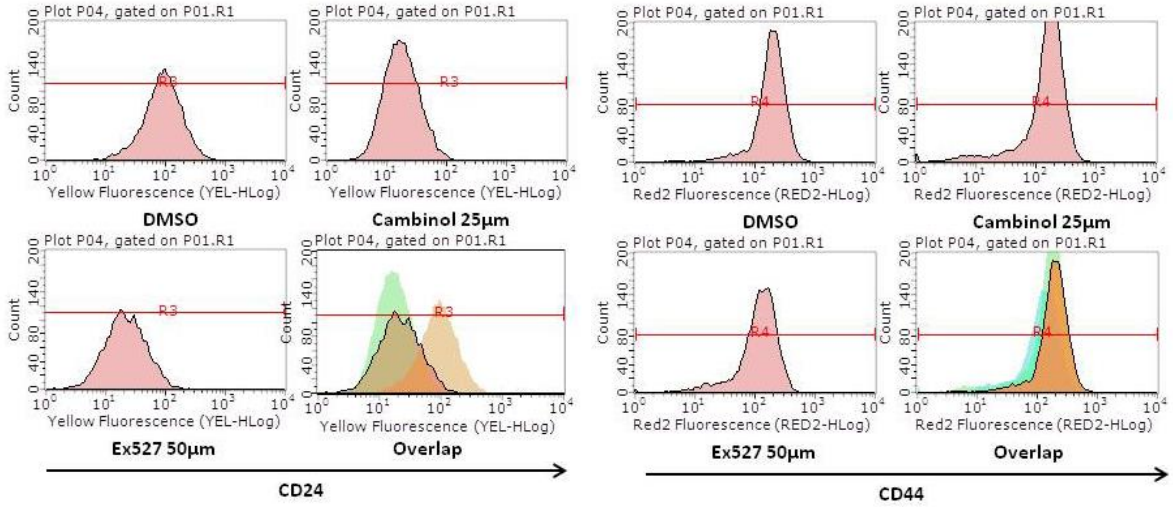


Figure 2. The histograph and the overlap of the CD24 intensity (2A) and the CD44 intensity (2B) in SIRT1 inhibitor treated cells comparing to DMSO control (orange-control; green-cambinol treated; blue-Ex527 treated) in MDA-MB-468 cells.

Adeflour for ALDH1a was performed on MDA-MB-231 cells. SIRT1 inhibitor cambinol treated cells showed dramatic decreased ALDH1a positive population, from 45% in the DMSO control to 6.9% in the cambinol 25 µM treated cells (Figure 3). T-47D cells had only minimal ALDH1a positive cells. Mammosphere assay was performed according the manufactory instruction. Because triple negative breast cancer MDA-MB-231 and MDA-MB-468 did not form tight and large spheres, T-47D cells were used for the assay. The mammosphere assay showed a significant reduce of sphere formation in T-47D cells when treated cells with SIRT1 inhibitor cambinol and Ex527.

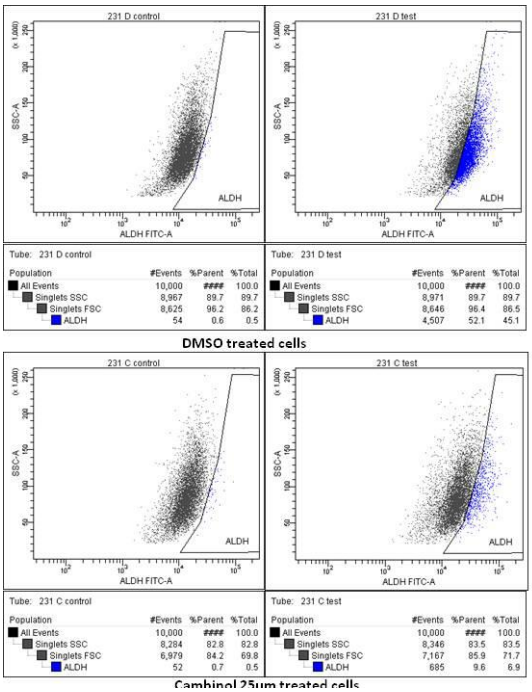
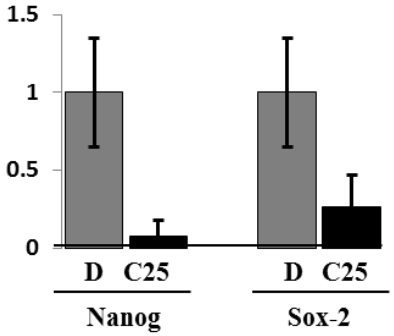
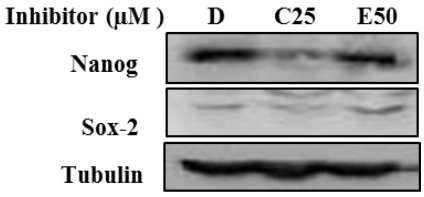


Figure 3. Breast cancer MDA-MB-231 cells were treated with DESO and SIRT1 inhibitor cambinol 25 µM for 24 hours. Cells were harvested and prepared according to the Adeflour manufactory instruction for the assay. For each sample, a half of the sample was inactived to serve as the sample negative control, and the ALDH1a positive cells were analyzed with flow cytometry. The DMSO treated MDA-MB-231 cells had 45% of ALDH1a positive cells, and the cambinol treated cells had only 6.9% ALDH1a positive cells. The study had been repeated for 3 times with the similar findings.

On the next step, cancer stem cell gene expression profile with SIRT1 inhibitor treatment using qRT-PCR was performed on several cell lines, and stem cell genes including Nanog and SOX-2 were analyzed. After cambinol treatment for 48 hours, MDA-MB-231 cells showed significantly gene expression down regulation for all genes (4A-B). T-47D cells showed similar findings (4C-D)



4A



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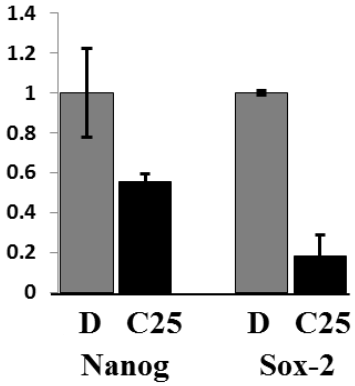
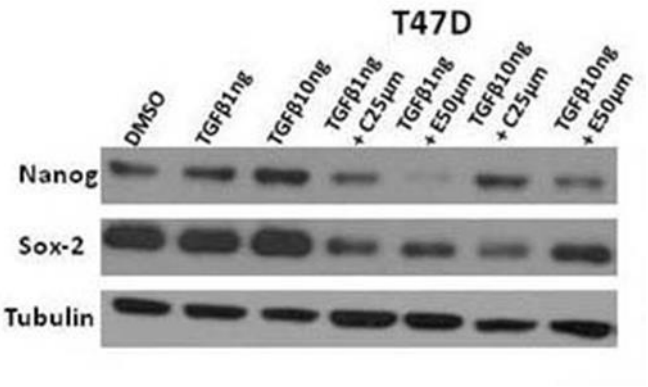


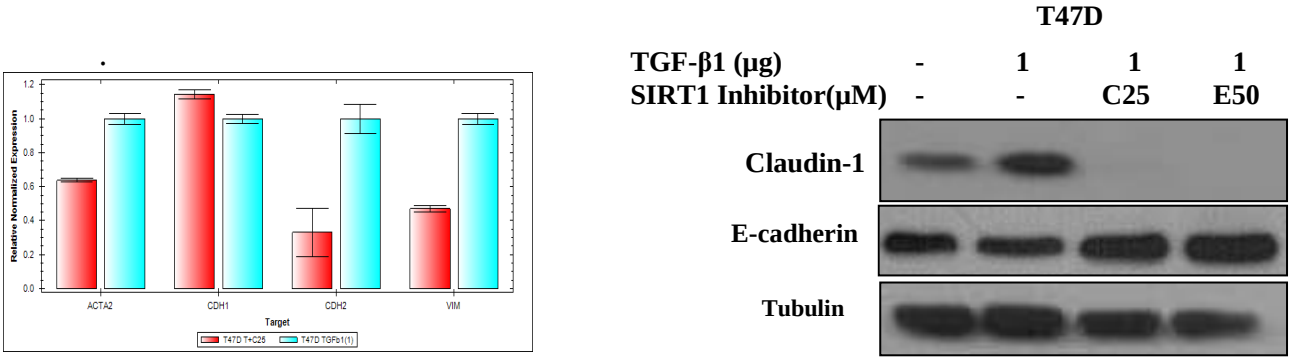


4C 4D

Figure 4. Inhibition of SIRT1 significantly reduced stem cell markers and associated genes in breast cancer. qRT-PCR was performed to detect stem cell associated genes Nanog and Sox-2 in MDA-MB-231(A) and T47D cells (C) with the treatment of either DMSO or 25μM of cambinol. Three similar experiments were performed, representative results were shown. Expressions of Nanog and SOX-2 by western blot were shown in MDA-MB-231 cells (B) and T47D cells (D).

T-47D cancer cells showed very good response to TGFβ1 stimulation compared to triple negative MDA-MB-231 cells, so T-47D was used to study for TGF β1 study. The western blot showed SIRT1 inhibitors cambinol and Ex527 also reduced the protein levels of Nanog and Sox-2 in T47D cells, corresponding to the qRT-PCR results. Furthermore, specifically knock down SIRT1 expression with SIRT1 small inhibitor RNA significantly reduced SOX-2 protein and slightly decreased Nanog protein in T-47D cells.

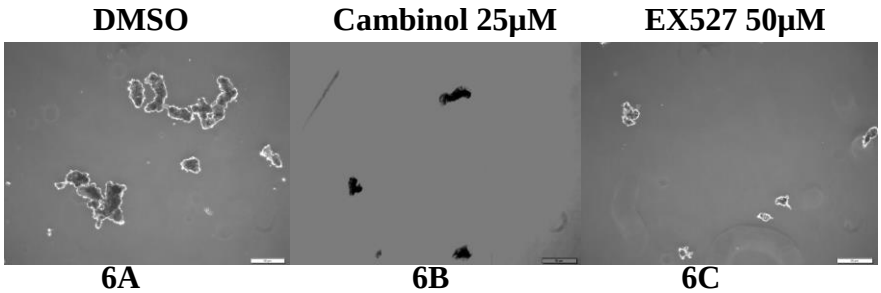
Since cancer cells with stem cell characters share some similar features with cancer cells undergoing EMT, we studied some molecular markers of EMT on the cancer cell lines. For cancer cells undergoing EMT, they loss E-cadherin and gain expression of N-cadherin, vimentin and smooth muscle actin. T-47D cells were stimulated with 1 ng/ml TGFβ1 with DMSO or cambinol 25 μM, and the gene expression was compared after 24 hours. Using qRT-PCR, SIRT1 inhibitor cambinol significantly blocked TGFβ1 induced vimentin, N-cadherin and SMA expression in T-47D breast cancer cells (5A). Using western blot, cambinol treatment significantly reduced claudin-1 and markedly increased E-cadherin, indicating cambinol blocking TGFβ1 induced EMT in T-47D cells (5B).



5A **5B**

Figure 5. SIRT1 inhibitor cambinol significantly blocked TGFβ1 induced EMT in T-47D breast cancer cells showing in both qRT-PCR (5A) and western blot (5B).

For further exploring SIRT1 inhibition influence stem cells, mammosphere functional assay had been performed. As shown in Figure 6, SIRT1 inhibitor cambinol and EX527 significantly reduced the mammosphere formation in T-47D cells.



6A **6B** **6C**

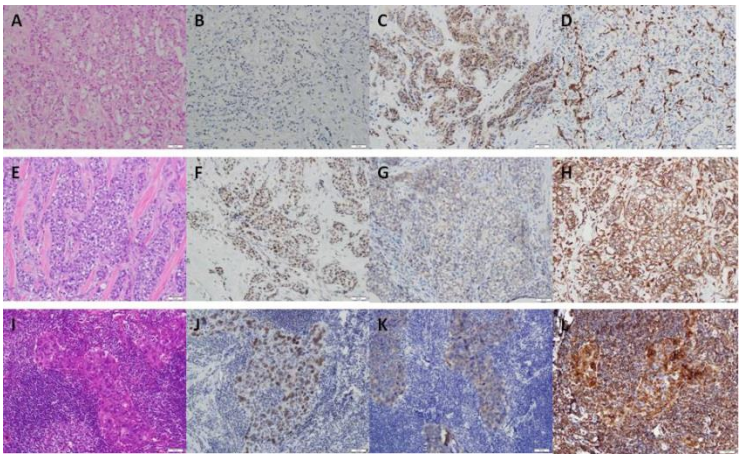
Figure 6. Inhibition of SIRT1 exhibited a decreased proportion and mammosphere formation capacity of breast cancer cells. T-47D was used for the mammosphere formation assay. Cells were cultured in conditional medium with DMSO, 25μm of cambinol and 50μm of Ex527 were added to the corresponding culture media. After 7 days in culture, mammospheres were counted and photographed.

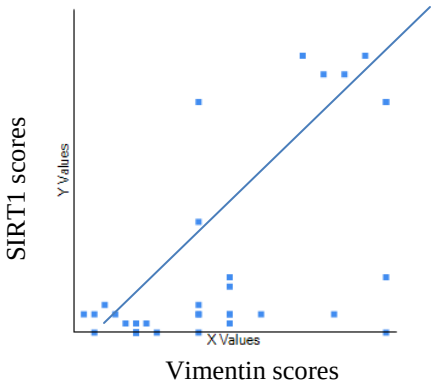
In summary, *in vitro* study in several breast cancer cell lines using flow cytometry analysis for CD24/CD44 expression and Adeflour for ALDH1a positive cells, functional study with mammosphere assay, and qRT-PCR for gene expression profile showed SIRT1 inhibitors

can induce cancer cell differentiation by reducing cancer stem cell population and blocking TGFβ1 induced EMT.

Task 2. Human breast cancer cells (from patient's samples) with CSC features have SIRT1.

32 breast cancers with variable grades and stages had been selected after the IRB approval, and SIRT1, several CSC markers (CD44, ALDH1a, SOX-2, OCT-4, Nanog and CD133) and EMT markers (vimentin, E-cadherin, Snail and Twist) had been used for immunohistochemistry. CD44/ALDH1a double staining has been used. All markers were blindly scored using H score formula, with staining intensity (0-3) times the cell percentage (0-100). The scores ranged from 0-300. Chi square and Pearson correlation coefficient were used for data analysis. The immnohistochemical results showed significant correlation between SIRT1 expression and breast cancer tumor grades, and grade 3 breast cancers had significantly high SIRT1 expression compared to grade 1/2 breast cancers (7A). Pearson correlation coefficient analysis showed significant positive correlation between SIRT1 and vimentin (p=0.0001) (7B). High grade breast cancers showed decreased E-cadherin, but the difference was not significant.





7M

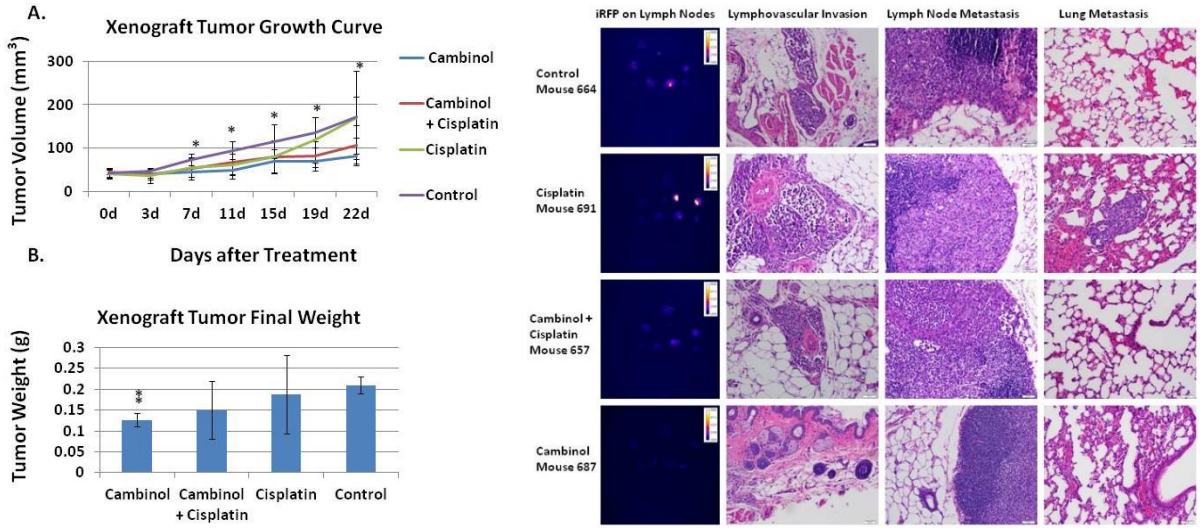
Figure 7. The representative H&E images from different grades carcinoma grade 1 (A), grade 2 (E) and grade 3 (I), the corresponding SIRT1 expression (B, F and J), E-cadherin (C, G, and K), and the viemntin (D, H and L). SIRT1 expression is positive related to the increasing vimentin expression in breast cancer samples (7M).

The correlations between the individual cancer stem cell marker showed no significant correlation with tumor grades. All 32 tested breast cancers were negative for OCT-4, and Nanog showed some cytoplasmic staining in some cases but no nuclear positivity. However, evaluation of 2 or more CSC markers showed that ALDH1a/CD44 coexpression/co-localization was significantly associated with high grade breast cancers (56% G3 vs 7% G1/2, p=0.007), and ALDH1a co-localization with one or more other markers (CD44, CD133 or SOX-2) also observed more in high grade cancers (72% G3 vs 21% G1/2, p=0.01). Double staining with ALDH1a/CD44 was performed in the human breast cancer tissue. ALDH1a/CD44 double positive cells are significantly associated with high-grade breast cancer (10/18 of G3 vs 1/14 of G1 and G2 cancer).

In summary, high grade breast cancers showed significant high SIRT1 expression, high vimentin expression, high percentage tumor cells positive for more cancer stem cells (especially double positive for ALDH1a/CD44).

Task 3. SIRT1 inhibition can decrease metastasis, induce differentiation of CSCs, reduce EMT, and increase tumor cell sensitivity to chemotherapy in xenograft mouse model.

The animal study protocol was approved by both UTHSC and DOD, the *in vivo* study was performed and whole body image with both PET and iRFP techniques by collaboration with Dr. Eva Sevick at the image core center, Institution of Molecular Medicine at UTHSC at Houston. A mammary fat pad cell injection was used to mimic the human breast cancer and hoped to observe the lymph node metastasis. The nude mice were used for MDA-MB-231 cell inoculation. MDA-MB-231 cells were used because it had been shown to have high metastatic potential and high chance of tumor formation than other breast cancer lines.

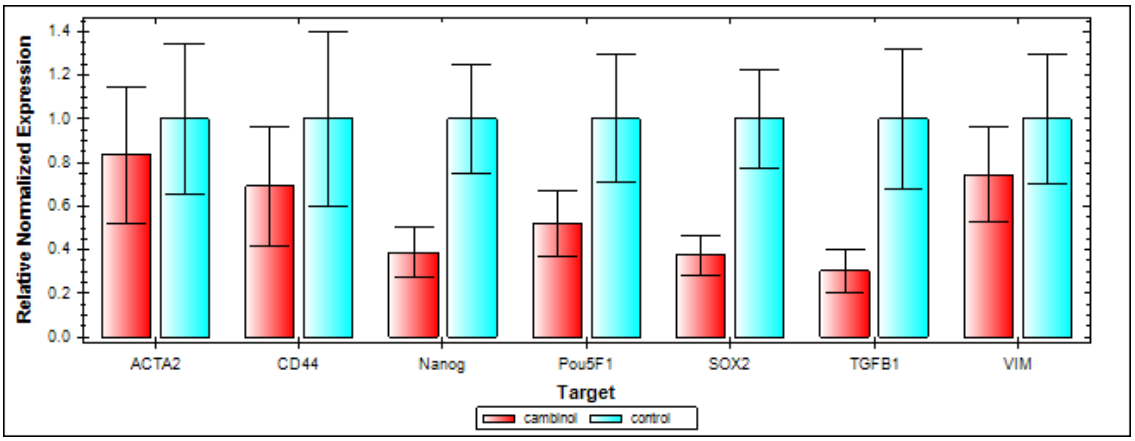


8C

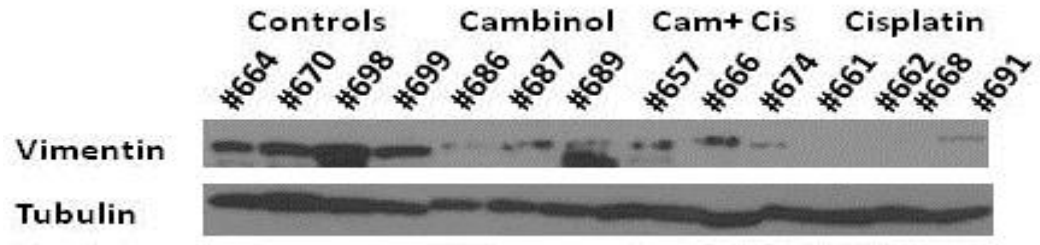
Figure 8. Xenograft tumor growth and metastasis using MDA-MB-231 cells. Figure 8A shows the tumor volume at different time points after MDA-MB-231 cell inoculation. Figure 8B shows the final tumor weights. Figure 8C shows the representative images of iRFP in lymph nodes, the lymphovascular invasion, the metastatic carcinoma in lymph nodes, and the lung tissue.

The *in vivo* study with MDA-MB-231 cells showed very good and exciting results. SIRT1 inhibitor cambinol treated mice (N=3) had significant slow tumor growth (indicated in the growth curve), and tumor volume showed minimal changes during the entire 3 weeks of therapy (8A). The DMSO control mice (N=5) had tumor volume tripled during the same period. Cambinol+cisplatin group (N=4) had similar tumor growth curve as the cambinol group with slightly large tumors. The cisplatin treated group (N=5) showed similar slow tumor growth curve at the first 2 week, but tumor started to grow very fast at the beginning of the 3rd week and reached to a similar tumor volume as the DMSO control group at the end. The growth curve indicated a gain of cisplatin resistance in animals treated with cisplatin only. When SIRT1 inhibitor cambinol was used together with cisplatin, the tumor remained in a very slow growth curve compared to the control group. This result is highly suggested that SIRT inhibitor cambinol could block the resistance of MDA-MB-231 cells to cisplatin. The final tumor weight showed significant small tumors in the SIRT1 inhibitor cambinol treated mice. The

intramammary fat pad inoculation of MDA-MB-231 cells generated a very good model to study the lymph node metastasis. Our study showed significant lymph node metastasis than blood stream metastasis (only one lung metastasis). Using iRFP whole body image and lymph node image study, positive lymph node metastasis was found in 5/5 control mice and 15/37 nodes, 4/5 cisplatin treated mice and 9/40 nodes, 3/4 cambinol+cisplatin treated mice and 5/40 nodes, and 0/3 cambinol treated mice and 0/20 nodes. H&E sections of skin showed marked lymphovacular invasion in the control mice but not in the cambinol treated mice.



9A

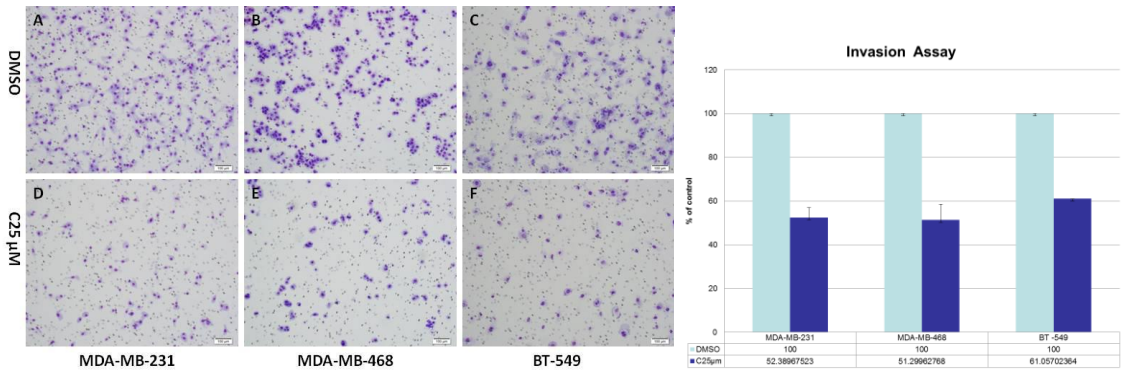


9B

Figure 9. Figure 9A shows the qRT-PCR results of down regulation of stem cell genes and EMT related genes in cambinol treated xenograft mice comparing to DMSO control mice. Figure 9B shows the western blot results of vimentin.

Using tumor tissue collected from the xenograft mice, qRT-PCR gene expression study showed significant down regulation of cancer stem cell genes (CD44, Nanog, Pou5F1 and SOX-2) and EMT genes (TGFβ1, vimentin and SMA) in cambinol treated mice compared to DMSO control mice (N=2) (9A). Western blot showed low vimentin protein levels in all treated groups compared with DMSO controls (9B).

SIRT1 inhibitors block breast cancer cell invasion was also confirmed in *in vitro* invasion assay Three triple negative breast cancer lines (MDA-MB-231, MDA-MB-468 and BT-549) were treated with cambinol 25 µM with DMSO as control. After 48 hours, the invasive cells were stained with Diff-Quik and counted. Cambinol treatment significantly blocked all 3 cancer cell invasion. Result showed cambinol dramatically blocked breast cancer cell invasion (Figure 10A). The percentage of invasion is 47.6%, 48.7% and 39.0% separately (Figure 10B).



10A 10B

Figure 10. The *In vitro* invasion assay showed that cambinol treatment significantly blocked iht invasion on all three triple negative breast cancer cells to 40-50%.

In summary, xenograft model with nude mice and MDA-MB-231 cells demonstrated that SIRT1 inhibitor cambinol decreased breast cancer growth, blocked cancer metastasis *in vivo* and invasion *in vitro*, and possible rescued cancer cells from resistance to cisplatin. Gene expression profile indicated SIRT1 inhibitor cambinol down regulated cancer stem cells *in vivo*. Our data supported SIRT1 inhibitor blocks EMT and reduced the differentiation of stem cells in xenograft tumor tissue.

Task 4. Wnt pathway is highly activated in breast CSCs and EMT of human breast cancer cell lines and specimens.

We investigated whether Wnt/β-caternin signaling has involved SIRT1 inhibition. There are various of target genes of Wnt pathway such as c-Myc, cyclinD1, and c-Jun. So we tested those Wnt pathway downstream genes using both MDA-MD-231 and T47D cells with cambinol (25μM) treatment. qRT-PCR results showed significant down regulation of cyclinD1, c-Myc and c-Jun (Figure 12 A and C), and the corresponding western blot showed the same findings (Figure 12 B and D). The results indicated SIRT1 inhibition blocked Wnt/β-catenin pathway.

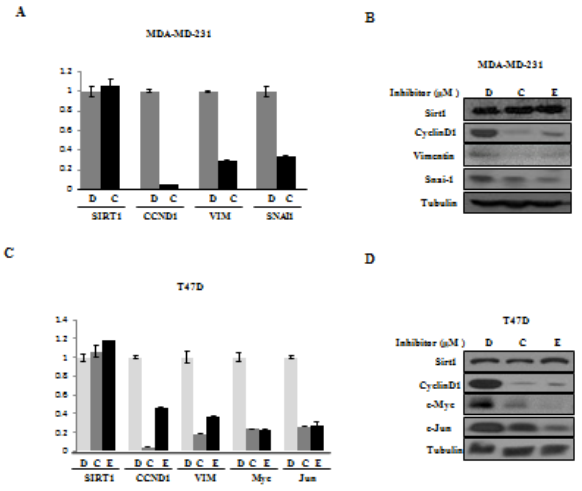
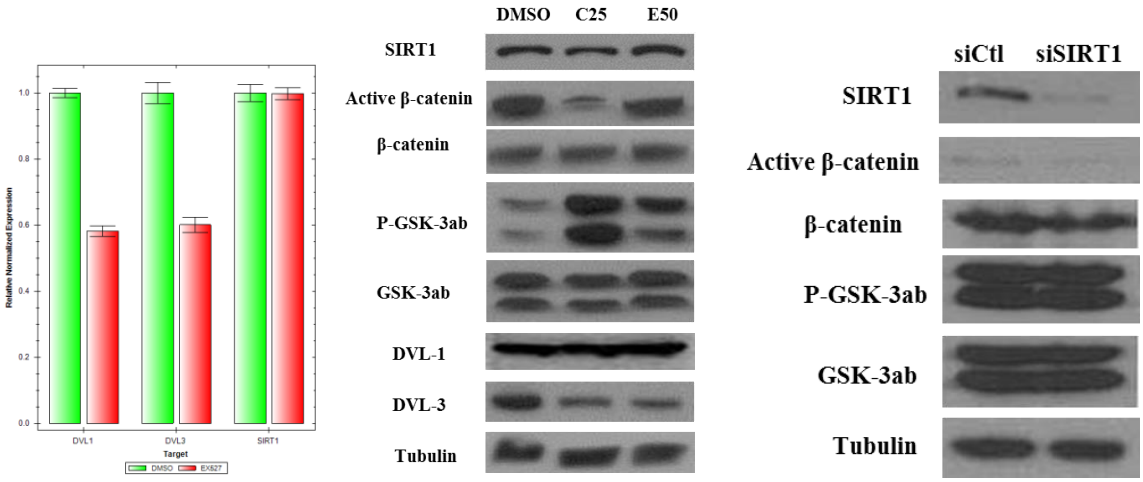


Figure 12. Wnt/β-caternin pathway is inhibited by SIRT1 inhibition with cambinol treatment. The qRT-PCR (Figure 12 A and C) and western blot (Figure 12 B and D) show the Wnt/β-caternin target genes including cyclinD1, c-Myc and c-Jun are significantly down regulated after the SIRT1 inhibition.

Dishevelled (DVL) is an element component in Wnt/β-catenine signals. Dvl proteins interact with other Wnt pathway proteins to form complex and conduct canonical and non-canonical Wnt cascade. The evidence of SIRT1 binding Dvl proteins to regulate Wnt/β-catenin in cancer

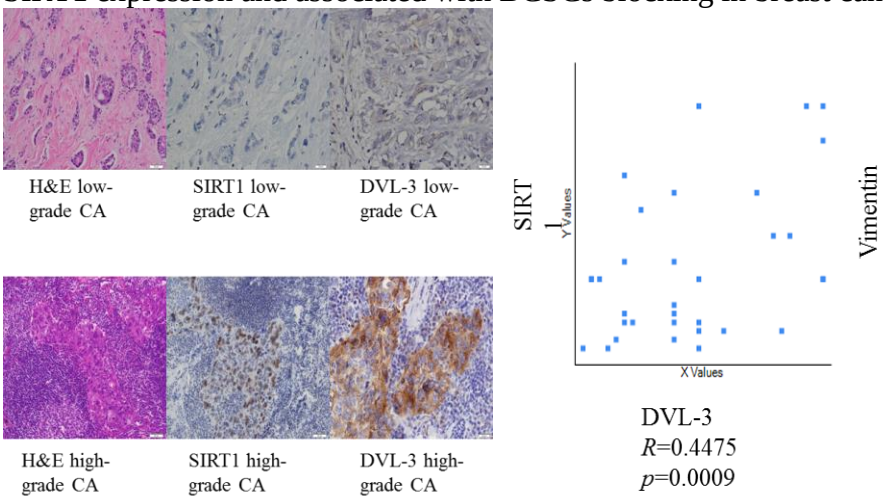
contexts has been revealed and the mechanism of regulation been investigated. We focus on analyzing the relationship of DVL-3 with SIRT1 in breast cancer. So we examined Dvl-3 expression levels in the existence of SIRT1 specify inhibitor Ex527. Result showed Ex527 block mRNAs of Dvl1 and Dvl3 other than SIRT1 itself (Figure 13A). As expected, DVL-3 was obviously down-regulated with the treatment of SIRT1 inhibitors cambinol and Ex527 in T47D cells (Fig13B) by western blot. These results concluded that SIRT1 inhibitors block Wnt/β-Catenin pathway via DVL-3.



A B C

Figure 13. SIRT1 inhibitor cambinol down regulates DVL-3 protein of Wnt/β-catenin pathway. Figure 13A shows the results of qRT-PCR, 13B shows the western blot of cambinol and EX527 treatment, and 13C shows the results of siRNA treatment.

Finally we examined whether DVL-3 expression is correlated with SIRT1 expresssion in breast cancer patients. Results showed DVL-3 expression is significantly high in high grade breast cancer (p=0.03 G1/2 vs G3), and DVL-3 expression is positively correlated with SIRT1 and Vimentin expression in tumor cells (Fig8A). These results concluded DVL-3 expression is significantly high in high grade breast cancer; DVL-3 expression is positively correlated with SIRT1 expression and associated with BCSCs blocking in breast cancers (Fig7B).



A B

Figure 14. Figure 14A shows the representative images of H&E, SIRT1 and DVL-3 in low grade and high grade breast cancer. Figure 14B shows the positive correlation between SIRT1 and DVL-3.

In summary, Wnt/β-caternin appears to be the pathway involving in SIRT1 regulation of cancer stem cells and EMT, and DVL-3 is the likely target of the regulation.

Task 5 . Wnt/β-catenin pathway is blocked in the SIRT1 inhibition xenograft tumor tissue, which is responsible for inducing differentiation of CSCs and reducing EMT.

Task 5 have been addressed in both task 3 and task 4.

Task 6. Using cell line *in vitro* study to demonstrate that SIRT1 regulates CSCs and EMT through activation of Wnt pathway via interaction with Dvl proteins.

As Figure 13 showed, DVL-3 and Wnt pathway were inhibited by SIRT1 inhibitor cambinol and EX527. For further elucidating the active β-catenin accumulated in cytoplasm or nuclear fraction in the breast cancer cells, we separated the lysate for cytoplasm or nuclear enrichment in T-47D cells (Figure 15). Cells were treated with different concentrations of cambinol, whole cell lysate (WL) was loaded as control amount of individual detected proteins. We found that SIRT1 existed in both cytoplasm fraction and nuclear fraction. Both the active and non-active β-catenin were down-regulated by SIRT1 inhibitor cambinol and the nuclear fraction of active β-catenin had the greatest decrease than the non-active β-catenin. Next, we examined the phosphorylated or whole GSK3ab, which is the main degrade factor of β-catenin in Wnt pathway, and results showed as expected that P-GSK3ab was up-regulated by the inhibition of SIRT1 in cambinol treatment (13B) but no significant changes in siRNA inhibition (13C).

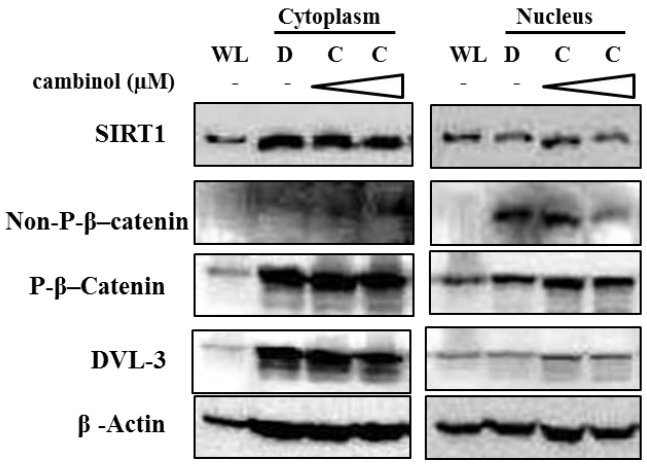
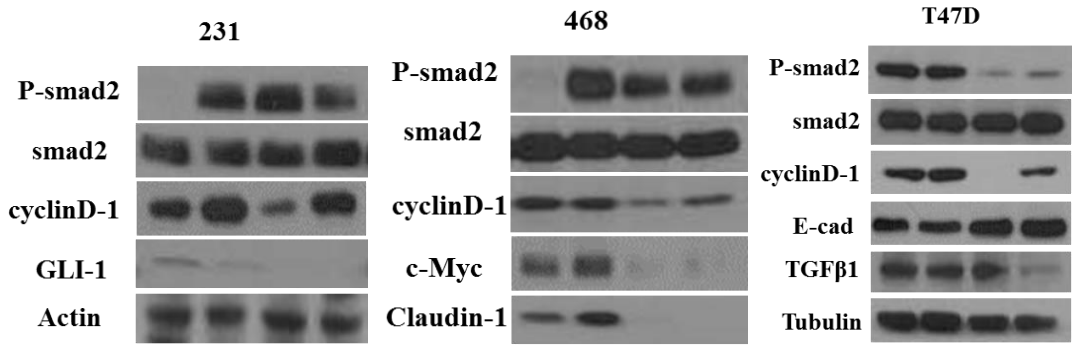


Figure 15. The cytoplasmic and nuclear distribution of active β-catenin (non-p-β-catenin), inactive β-catenin (p-β-catenin) and DVL-3 in T47-D cells.

In summary, SIRT1 inhibitors are likely regulated Wnt pathway by regulation of DVL3 protein.

We also explored the possibility of the role of SIRT1 regulation of TGFβ1 pathway in the EMT signal transduction and stem cell associated genes. We found that SIRT1 clearly regulated TGFβ1activity (Figure 16), which is a novel finding, and further study will be performed.



16A 16B 16C

Figure 16. SIRT1 inhibition down regulates TGFβ1 pathway in breast cancer cells.

4. Key Research Accomplishments

A.

Using 32 breast cancer samples, SIRT1 expressions is high in high grade of breast cancers, high SIRT1 expression is associated with cancer EMT, and high SIRT1 expression is associated with cancer stem cells with ALDH1a/CD44 double stain technique. The results support SIRT1 expression is associated with cancer stem cell features.

B.

Breast cancer stem cell markers $CD24^{low}/CD44^{high}$ and ALDH1a are highly expressed in high grade, triple negative breast cancer cell lines MDA-MB-231 and MDA-MD-468, and inhibition of SIRT1 with inhibitor cambimol and EX527 significantly reduced the stem cell population in the triple negative breast cancer. Mammosphere functional assay shows SIRT1 inhibitors significantly reduce mammosphere formation in T-47D cells. Our results indicate that inhibition of SIRT1 can induce differentiation of CSCs in breast cancer cell lines.

C.

SIRT1 inhibitor can inhibit tumor growth, metastasis, and rescue drug resistance in xenograft mouse model with MDA-MB-231 cells, and SIRT1 inhibitors can block breast cancer cell invasion in several triple negative breast cancers. Our results indicate that SIRT1 possesses a potential therapeutic target in high grade and high stage breast cancer.

D.

Wnt/β-catenin pathway is regulated by SIRT1. SIRT1 inhibition can significantly reduce β-catenin protein especially active β-catenin in the nuclei, and significantly reduce the Wnt pathway downstream genes such as cyclinD1 and c-Myc. DVL-3 protein appears to be the regulation protein involved in Wnt pathway. DVL-3 is highly correlated in breast cancer samples by immunohistochemistry. These data indicated SIRT1 is significantly associated with cancer stem cell Wnt pathway. Our limited data also indicates that TGFβ pathway, another cancer stem cell pathway, is regulated by SIRT1.

In summary, our study demonstrate that SIRT1 plays an important role in breast cancer stem cells, and our results provide some clear evidences that SIRT1 is a potential therapeutic target in breast cancer.

5. Conclusion

The role of SIRT1 in tumorigenesis remains controversial, i.e. whether it acts as a tumor promoter or a tumor suppressor, and the role of SIRT1 in breast cancer stem cells has not been

well explored. SIRT1 is strongly expressed in embryonic stem cells, and SIRT1 downregulation is necessary to establish correct and specific cell differentiation. Cancer stem cells may serve as cancer reservoir for breast cancer recurrence and distant metastasis. One significant challenge of cancer stem cell study is to identify possible stem cells with specific markers. The current study has been designed to study the import and critical issues about finding the cure for breast cancer by targeting the cancer stem cells.

Over the three years period, we achieved significant understanding about the role of SIRT1 in breast cancer stem cells. Our results show that SIRT1 is an important regulator of breast cancer stem cells and SIRT1 inhibition can significant reduce cancer stem cells and lead cancer cell differentiation. SIRT1 inhibition can block tumor cell growth, invasion and metastasis, and can rescue drug resistance. SIRT1 inhibition can block cancer cell to epithelial mesenchymal transition and block the cancer metastasis. Our data suggest that the important signal transduction pathway of Wnt/β-catenin and TGFβ is highly regulated by SIRT1.

The significant findings of the current study prove that SIRT1 can be used as a therapeutic target, especially in high grade and metastatic breast cancer with some small molecular weights of SIRT1 inhibitors such as cambinol or EX527. The future study includes subclincal trial using SIRT1 inhibitors on primary tumor tissue and primary tumor xenograft model, using SIRT1 inhibitors in CSF metastatic mouse model, and using SIRT1 inhibitors in locally advanced breast cancer.

6. Publications, Abstracts, and Presentations

Publications:

1. Zhu B, Ronbinson H, Zhang S, Wu G, Sevick E. Longitudinal far red gene-reporter imaging of cancer metastasis in preclinical models: a tool for accelerating drug discovery. Biomedical Optics Express. 6:3341-51, 2015. PMID: 26417506

2. Sang M, Hulsurkar M, Zhang X, Song H, Zheng D, Zhang Y, Li M, Xu J, Zhang S, Ittmann M, Li W. GRK3 is a direct target of CREB activation and regulates neuroendocrine differentiation of prostate cancer cells. Oncotarget 2016. PMID: 27191986

Abstracts:

1. Songlin Zhang, Min Li, Baoxiang Guan, Robert Brown. SIRT1 inhibitors significantly reduce cancer stem cells and block epithelial mesenchymal transition in breast cancer cells. 2014 San Antonio Breast Cancer Symposium, San Antonio.
2. Frances Compton, Min Li, Songlin Zhang. High SIRT1 expression associated with epithelial mesenchymal transition in breast cancer. 2015 ASCP Annual Scientific Meeting, Long Beach.
3. Frances Compton, Min Li, Songlin Zhang. Cancer stem cell marker expression associated with high grade breast cancer. 2015 CAP Annual Meeting, Chicago.

Presentation:

1. SIRT1 as a therapeutic target in breast cancer. Department Research Seminar, UTHSC, Houston 03/21/2014
2. SIRT1 as a therapeutic target in breast cancer. Grand Round, medical oncology, UTHSC, 09/15/2014

7. Inventions, Patents and Licenses

Nothing to report

8. Reportable Outcomes

Nothing to report

9. Other Achievements

Another grant was submitted for subclinical study.

10. References

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11. Appendices

None.