Publications

Clinics of Oncology

Review Article

Triple-Negative Breast Cancer: Is it Possible to Reverse Receptor Negativity and Regain Sensitivity to Receptor-Targeting Therapies?

Huimei Yi[1,2], Lu Lu[1,2], Hui Yao[1,2], Guangchun He[1,2], Shichao Yan[1,2], Chao Chen[1,2], Mi Wu[1,2], Qiuting Zhang[1], Guifei Li[1,3], Xiyun Deng[1, 2*]

[1]Department of Translational Cancer Stem Cell Research, Hunan Normal University, China

[2]Departments of Pathology and Pathophysiology, Hunan Normal University School of Medicine, China

[3]Department of Nursing, Hunan Normal University School of Medicine, China

Volume 1 Issue 4- 2018
Received Date: 17 July 2018
Accepted Date: 07 Aug 2018
Published Date: 14 Aug 2018

1. Abstract

Effective treatment of Triple-Negative Breast Cancer (TNBC) is lacking due to the absence of Estrogen Receptor (ER), Progesterone Receptor (PR) and human epidermal growth factor receptor 2 (HER2). The recent study showed that inactivation of ER is associated with hypermethylation of the ER promoter, MAPK signaling pathways, estrogen withdrawal, and hypoxia. Therefore, simultaneous inhibition of MAPK, NF-κB/RhoC signaling pathways may reverse the status of ER and regain the sensitivity to anti-estrogen drugs such as tamoxifen. In addition, our research indicated that lovastatin, a natural 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor, induced the reexpression of HER2 and possibly ER, although the localization of these reexpressed HER2 is different from the prototype HER2 in HER2-positive cells. Here, we discuss about the possibility of reversing hormone receptors and HER2 receptor and regaining sensitivity to receptor-targeting drugs. This possibility provides a valuable opportunity and a novel strategy for the precision therapy of the difficult-to-treat disease of TNBC.

2. Keywords

Triple-negative breast cancer; Reversal; Caner therapy; Lovastatin

3. Introduction

Breast cancer is the most common female malignancy and is responsible for about 14% of cancer-related deaths in women [1]. Triple-negative breast cancer (TNBC), characterized by the absence of expression of Estrogen Receptor (ER), Progesterone Receptor (PR), and human epidermal growth factor receptor 2 (HER2), is the most aggressive and deadly subtype of breast cancer [2-4]. TNBCs preferably strike young patients and constitute 12-24% of all diagnosed breast cancer cases. Tumor characteristics of TNBCs include rare histologies, high grade, elevated mitotic count, tumor necrosis, pushing margins of invasion, larger tumor size and axillary node involvement [5]. Because of the absence of these receptors, currently, TNBC patients cannot be treated with endocrine or HER2-targeting therapies used for non-TNBC patients [6]. For many years, chemotherapy has been the mainstay of treatment for TNBC. Unfortunately, although some of TNBCs are initially chemosensitive, prognosis remains poor in TNBCs because these patients relapse more frequently and more aggressively than hormone receptor-positive breast tumors [5]. Therefore, understanding of the biology of these receptors, e.g., the mechanisms by which their expressions get lost, the possibilities of reactivation of their expressions and regaining sensitivity to receptor-targeting therapies, becomes extremely crucial both on the bench and in the clinic.

4. Mechanisms of ER Gene Inactivation and Reactivation

It has been well established that a subset of breast cancer cells exhibit hypermethylation of the ER promoter, which results in repression of the transcription of the ER gene [7,8]. In addition, ER-negative breast cancer cells are subject to hyperactivation of mitogen-activated protein kinase (MAPK) signaling due to overexpression of epidermal growth factor receptor (EGFR) or c-erbB-2 (the gene coding for HER2) [9], estrogen withdrawal [10], and hypoxia [11]. These events all contribute to the lack

***Corresponding Author (s):** Xiyun Deng, Department of Translational Cancer Stem Cell Research, Hunan Normal University, China, E-mail: dengxiyunmed@hunnu.edu.cn*

Citation: Huimei Yi, Lu Lu, Hui Yao, Guangchun He, Shichao Yan, Chao Chen, Mi Wu, Qiuting Zhang, Guifei Li, Xiyun Deng, Triple-Negative Breast Cancer: Is it Possible to Reverse Receptor Negativity and Regain Sensitivity to Receptor-Targeting Therapies? Clinics of Oncology. 2018; 1(4): 1-4.

of ER expression in ER-negative breast tumors. In those cells in which the ER promoter has been silenced by DNA methylation, inhibition of DNA methylation by 5-aza-2'-deoxycytidine [12] and/or inhibition of histone deacetylation by panobinostat [13] may lead to reactivation of the ER promoter and thus re-expression of the ER gene. Correspondingly, repression of ER expression by MAPK hyperactivation can be reversed via direct inhibition of MAPK by small molecule inhibitors such as U0126. Inhibition of the signaling events upstream of MAPK such as the EGFR and HER2 pathways by Iressa and Herceptin, respectively, may also lead to reversal of ER negativity [9].

4.1. ER reexpression restores sensitivity to antiestrogens

Importantly, it should be noted that the reversal of ER expression restores responses of the breast cancer cells to antiestrogens such as tamoxifen and fulvestrant (trade name: Faslodex, also called ICI 182,780) in *in vitro* studies. However, in TNBC cells such as SUM 102 and SUM 159, inhibition of MAPK signaling does not lead to reexpression of ER. Furthermore, in another TNBC cell line, SUM 149, reexpression of ER induced by MAPK inhibition does not lead to restoration of responses to antiestrogens. This is possibly because of hyperactivation of the NF-κB and RhoC signaling pathways [14]. Thus, although the repression of ER expression by MAPK hyperactivation is operative in these cells, they have additional mechanisms, e.g., activation of NF-κB and RhoC signaling pathways that allow them to bypass restored ER signaling and remain resistant to antiestrogen therapies. Therefore, three possibilities may exist. First, in some breast cancer cells, ER expression can be restored and sensitivity to antiestrogens regained. Second, in some TNBC cells, even after ER expression is restored, sensitivity to antiestrogens cannot be regained. Third, in some other TNBCs, it's difficult to restore ER expression and regain sensitivity to antiestrogens. We need to keep in mind that in order for these TNBC cells to regain sensitivity to antiestrogens, it might be necessary to simultaneously apply dual and/or multiple suppression of signaling pathways important for cell growth and/or survival, such as AMPK signaling and NF-κB/RhoC signaling.

Estrogen-related receptor α (ERRα) is a structural homolog of ER. However, ERRα is functionally distinct from ER [15]. ERRα is a master regulator of cellular energy metabolism and a critical regulator of cancer development because it can accommodate energy demands of proliferating cancer cells [16-18]. The expression of ERRα tends to be inversely correlated with ER/PR expression in breast cancer and is essential for the growth of TNBC [16,19]. In these cells, ERRα negatively regulates the expression of S6K1, a key enzyme responsible for the global protein synthesis, and downregulation of ERRα sensitizes TNBC cells to mTORC1/S6K1 inhibitors [20]. These observations suggest that dual inhibition of ERRα and mTORC1/S6K1 signaling may have clinical utility in the treatment of TNBC.

4.2. Lovastatin: a novel reverser of the ER/HER2 negative phenotype in TNBC?

Lovastatin (LV), a natural compound, is one of the most common lipid-lowing drugs in clinic [21]. Over the recent decades, it has been shown that LV can inhibit proliferation and induce the differentiation or apoptosis of breast cancer cells [22]. Our previous studies have indicated that LV can inhibit the stemness characteristics of TNBC *in vivo* and *in vitro* [23,24]. Furthermore, we found LV induced the reexpression of ER and HER2 in a nude mouse model of orthotopic tumor growth of TNBC MDA-MB-231 Cancer Stem Cells (CSCs) in the mammary fat pad **(Figure 1)**. Moreover, in cultured TNBC CSCs, LV-induced expression of HER2 was confirmed by laser scanning confocal microscopy. There was no increase of HER2 expression in the HER2-positive MDA-MB-453 CSCs **(Figure 2)**. However, unlike the membranous distribution on non-TNBC cells, the reexpressed HER2 in TNBC cells has diffuse cytoplasmic distribution. This suggests that LV-induced HER2 in TNBC cells might be different from the prototype HER2 in HER2-positive cells. Whether this induced HER2 could sensitize the TNBC CSCs to HER2-targeting drugs such as Herceptin is currently not known and is worthy of further investigation.

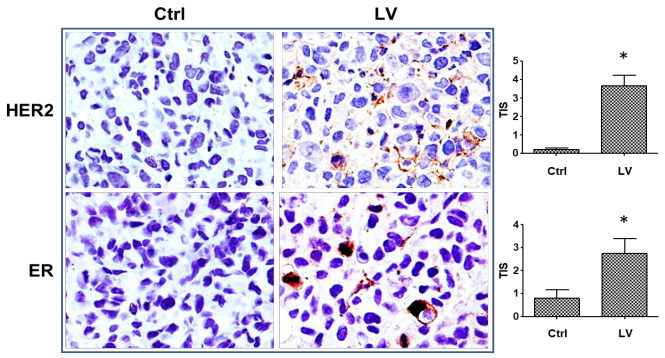

Figure 1: Lovastatin reverses the HER2/ER negative phenotype in orthotopic tumor growth of TNBC cancer stem cells *in vivo*. Representative images showing immunohistochemical staining of HER2 and ER in the nude mouse model of orthotopic tumor growth of MDA-MB-231 cancer stem cells. LV-induced HER2 and ER showed membranous and nuclear staining, respectively. *: $P < 0.05$. Original magnification: 400 ×. TIS: total intensity score.

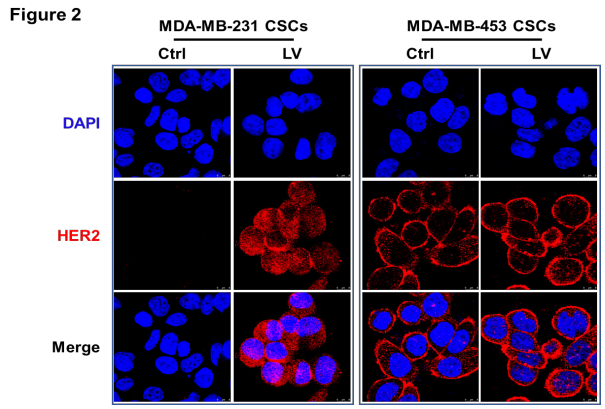

Figure 2: Lovastatin induces the reappearance of HER2 in TNBC cancer stem cells *in vitro*. Representative images showing immunofluorescence staining of the expression and localization of HER2. MDA-MB-231 (TNBC) and HER2-positive cancer stem cells were treated with LV or vehicle control for 48 h. HER2 immunofluorescence (red) was observed by laser scanning confocal microscopy. DAPI was used to stain the nucleus (blue). Scale bar = 8 μm. Original magnification: 630 ×.

5. Perspectives

Although not so evident, pieces of evidence suggest that the receptor-negative phenotype can be reversed in TNBC. It is hoped that reappearance of these missing receptors will have a great impact on the clinical treatment of TNBC. For example, the re-expressed ER can be targeted by antiestrogen therapies as mentioned above. In addition, pegvisomant (trade name: Somavert), a growth hormone antagonist, may also be able to inhibit ER-positive breast cancers [25]. In a similar way, TNBC tumors re-expressing HER2 can be treated by targeted therapies such as receptor kinase inhibitors such as Herceptin (Trastuzumab) and Lapatinib [26,27].

In despite of the possibility of reversing receptor negativity and regaining chemosensitivity, we should not be too optimistic about its clinical implications. Two important issues should be kept in mind. First, reversal of receptor negativity is only part of the story. The completeness of whole story depends on another critical parameter, i.e., whether reappearance of the receptor sensitizes the cells to the targeted therapies. The latter is largely influenced by the existence of other growth and/or survival-promoting signaling pathways, such as the NF-κB and RhoC pathways. In an ideal scenario, dual or even multiple inhibitions of the complex intracellular signaling events, e.g., simultaneous targeting of the receptor pathway, the NF-κB pathway, as well as other growth- and/or survival-related signal pathways, might be beneficial. Second, although reexpressed receptors might confer enhanced sensitivity to receptor-targeting therapies, the impact of these reappeared receptors on cell proliferation and/or survival is not known. Since HER2 and ER, *per se*, are components of intracellular signaling pathways, which are related to cell proliferation and/or survival. Therefore, we have to investigate the impact of the reversal of receptor negativity on cell behavior in the long run. We are far away from a clear view of the whole picture of the reversal of receptor negativity. Nevertheless, this possibility provides a valuable opportunity for us to fight against the difficult-to-treat disease of TNBC.

References

1. de Groot AF, Kuijpers CJ, Kroep JR. CDK4/6 inhibition in early and metastatic breast cancer: A review. Cancer Treat Rev. 2017;60:130-8.

2. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61-70.

3. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, et al. Cancer treatment and survivorship statistics, 2014. CA Cancer J Clin. 2014;64(4):252-71.

4. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363:1938-48.

5. Bosch A, Eroles P, Zaragoza R, Vina JR, Lluch A. Triple-negative breast cancer: molecular features, pathogenesis, treatment and current lines of research. Cancer Treat Rev. 2010;36(3):206-15.

6. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol. 2007;8(3):235-44.

7. Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE. Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. Cancer Res. 1994;54(10):2552-5.

8. Lapidus RG, Nass SJ, Butash KA, Parl FF, Weitzman SA, Graff JG, et al. Mapping of ER gene CpG island methylation-specific polymerase chain reaction. Cancer Res. 1998;58(12):2515-9.

9. Oh AS, Lorant LA, Holloway JN, Miller DL, Kern FG, El-Ashry D. Hyperactivation of MAPK induces loss of ERalpha expression in breast cancer cells. Mol Endocrinol. 2001;15:1344-59.

10. Jeng MH, Yue W, Eischeid A, Wang JP, Santen RJ. Role of MAP kinase in the enhanced cell proliferation of long term estrogen deprived human breast cancer cells. Breast Cancer Res Treat. 2000;62:167-75.

11. Kronblad A, Hedenfalk I, Nilsson E, Pahlman S, Landberg G. ERK1/2 inhibition increases antiestrogen treatment efficacy by interfering with hypoxia-induced downregulation of ERalpha: a combination therapy potentially targeting hypoxic and dormant tumor cells. Oncogene. 2005;24(45):6835-41.

12. Ferguson AT, Lapidus RG, Baylin SB, Davidson NE. Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression. Cancer Res. 1995;55(11):2279-83.

13. Zhou Q, Atadja P, Davidson NE. Histone deacetylase inhibitor LBH589 reactivates silenced estrogen receptor alpha (ER) gene expression without loss of DNA hypermethylation. Cancer Biol Ther. 2007;6(1):64-9.

14. Bayliss J, Hilger A, Vishnu P, Diehl K, El-Ashry D. Reversal of the estrogen receptor negative phenotype in breast cancer and restoration of antiestrogen response. Clin Cancer Res. 2007;13(23):7029-36.

15. Jarzabek K, Koda M, Kozlowski L, Sulkowski S, Kottler ML, Wolczynski S. The significance of the expression of ERRalpha as a potential biomarker in breast cancer. J Steroid Biochem Mol Biol. 2009;113(1-2):127-33.

16. Stein RA, Chang CY, Kazmin DA, Way J, Schroeder T, Wergin M, et al. Estrogen-related receptor alpha is critical for the growth of estrogen receptor-negative breast cancer. Cancer Res. 2008;68(21):8805-12.

17. Deblois G, Giguere V. Oestrogen-related receptors in breast cancer: control of cellular metabolism and beyond. Nat Rev Cancer. 2013;13(1):27-36.

18. Deblois G, St-Pierre J, Giguere V. The PGC-1/ERR signaling axis in cancer. Oncogene. 2013;32(30):3483-90.

19. Manna S, Bostner J, Sun Y, Miller LD, Alayev A, Schwartz NS, et al. ERRalpha Is a Marker of Tamoxifen Response and Survival in Triple-Negative Breast Cancer. Clin Cancer Res. 2016;22(6):1421-31.

20. Berman AY, Manna S, Schwartz NS, Katz YE, Sun Y, Behrmann CA, et al. ERRalpha regulates the growth of triple-negative breast cancer cells via S6K1-dependent mechanism. Signal Transduct Target Ther. 2017;2.

21. Yang T, Liu J, Luo F, Lin Q, Rosol TJ, Deng X. Anticancer properties of Monascus metabolites. Anticancer Drugs. 2014;25(7):735-44.

22. Campbell MJ, Esserman LJ, Zhou Y, Shoemaker M, Lobo M, Borman E, et al. Breast cancer growth prevention by statins. Cancer Res. 2006;66(17):8707-14.

23. Song L, Tao X, Lin L, Chen C, Yao H, He G, et al. Cerasomal Lovastatin Nanohybrids for Efficient Inhibition of Triple-Negative Breast Cancer Stem Cells To Improve Therapeutic Efficacy. ACS Appl Mater Interfaces. 2018;10(8):7022-30.

24. Yao H, He G, Yan S, Chen C, Song L, Rosol TJ, et al. Triple-negative breast cancer: is there a treatment on the horizon? Oncotarget. 2017;8(1):1913-24.

25. Divisova J, Kuiatse I, Lazard Z, Weiss H, Vreeland F, Hadsell DL, et al. The growth hormone receptor antagonist pegvisomant blocks both mammary gland development and MCF-7 breast cancer xenograft growth. Breast Cancer Res Treat. 2006;98(3):315-27.

26. Nakanishi T, Chumsri S, Khakpour N, Brodie AH, Leyland-Jones B, Hamburger AW, et al. Side-population cells in luminal-type breast cancer have tumour-initiating cell properties, and are regulated by HER2 expression and signalling. Br J Cancer. 2010;102(5):815-26.

27. Roberts PJ, Usary JE, Darr DB, Dillon PM, Pfefferle AD, Whittle MC, et al. Combined PI3K/mTOR and MEK inhibition provides broad antitumor activity in faithful murine cancer models. Clin Cancer Res. 2012;18(19):5290-303.