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LETTER ARTICLE

Lapatinib as a Dual Tyrosine Kinase Inhibitor Unexpectedly Activates Akt in MDA-MB-231 Triple-Negative Breast Cancer Cells

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Abstract: ***Background*:** MDA-MB-231 is a Triple-Negative Breast Cancer (TNBC) cell line, which is resistant to tyrosine kinase inhibitors, such as lapatinib. Lapatinib is well-recognized as an anti-EGFR and anti-Her2 compound. Here, we report one of the possible explanations for lapatinib-resistance in TNBC cells, the most incurable type of breast cancer.

***Methods*:** Using western blotting, we have observed that lapatinib-treated cells enhanced activation of Akt, an oncogenic protein activated at downstream of EGFR signaling.

***Results*:** Anti-EGFR activity of Lapatinib would be counteracted with sustained activation of Akt. We found lapatinib-resistance in TNBC can be managed by administering Akt inhibitors. Further, lapatinib enhanced PI3K/Akt signaling is an alternative pathway to ensure the viability of MDA-MB-231 cells. There might also be unknown targets for lapatinib, which needs further investigation.

***Conclusion*:** This observation opens up a new discussion on overcoming resistance to tyrosine kinase inhibitors, a key challenge in treating TNBC.

Keywords: Breast Cancer, TNBC, Lapatinib, Akt, Resistance, EGFR.

1. DEAR EDITOR

Beretta (2019) has discussed the advances made in molecular oncology of drug resistance [1]. Tang and Ling (2014) also described the effects of aberrant activation of PI3K/Akt/mTOR pathway on drug resistance in prostate cancer [2]. Epidermal growth factor receptor (EGFR), which is a well-known tyrosine kinase receptor, is expressed in 50% of Triple-Negative Breast Cancer (TNBC) cells [3]. Although chemotherapy using Tyrosine Kinase Inhibitors (TKIs) has been recognized as one of the important therapeutic methods against TNBCs, these patients, unfortunately, have developed resistance to TKIs and EGFR targeted therapy [4]. Recently, we studied the antitumor effects of berberine in MDA-MB-231 and MCF-7 cells. It was observed that MDA-MB-231 TNBC cells showed resistance to berberine and lapatinib compared with MCF-7, an estrogen receptor (ER)-positive ductal carcinoma cell line [5].

Here, we discuss our recent finding that showed lapatinib increased the activation of Akt in the MDA-MB-231 TNBC cell line. We did not observe this with another compound, berberine, in the same cell line with the same genetic back ground. Akt is an oncogenic protein located at the downstream of EGFR signaling. The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) is required to generate phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and recruit Akt from the cytoplasm to plasma membrane. The 3-phosphoinositide-dependent protein kinase-1 (PDPK1) and the mammalian target of rapamycin complex 2 (mTORC2) are both required to phosphorylate and fully activate Akt (overactivation of Akt impedes EGFR targeted therapy). We observed that lapatinib greatly increases the level of Akt activation compared with untreated MDA-MB-231 cells. We repeated the experiments twice to bolster our findings. Since lapatinib is an EGFR inhibitor, we observed a reduced level of p-EGFR but an increased level of p-Akt when the cells were treated with lapatinib [5]. There may be unknown targets for lapatinib, which can directly or indirectly affect Akt.

Fig. (**1**) shows that lapatinib enhances Akt activation in two independent experiments performed on MDA-MB-231 cell cultures. Treatment with berberine did not activate Akt suggesting that it is a better option for treating TNBC cells in the case of lapatinib resistance. Activation of Akt after lapatinib treatment has not been directly reported. There may be many reasons for this, such as increased passage number of MDA-MB-231 cells which increases the chance of collecting

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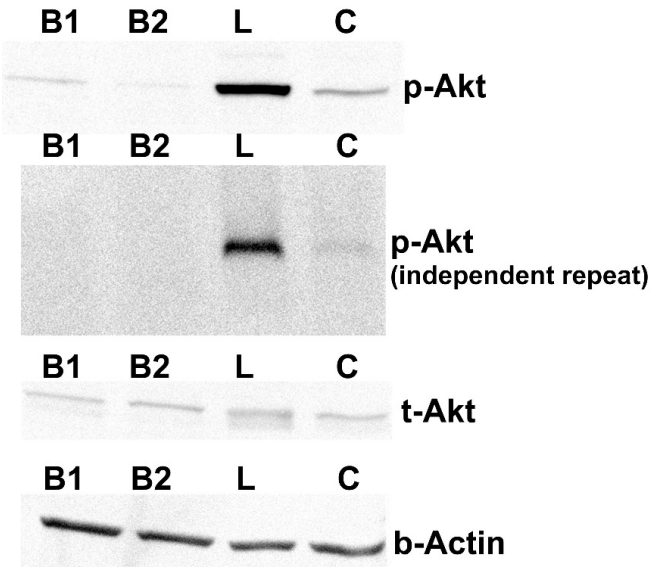

Fig. (1). The Effects of lapatinib and berberine on p-Akt in MDA-MB-231 cells. Two independent cell cultures were used for lapatinib (L)-, berberine (B1 & B2)-, and DMSO-treated (C) (as negative control) cultures. Western blot of p-Akt for each independent flask was performed in duplicate. It was observed that lapatinib enhanced Akt activation, but, in contrast, berberine acted as an Akt inhibitor in two different concentrations. Western blots of the total Akt and β-Actin are also demonstrated. For more information refer to our previous article (doi: 10.1016/j.pharep.2018.07.005; [5]). B1: 12.5 μM, B2: 25 μM, L: 50 μM, and C: 1% DMSO. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

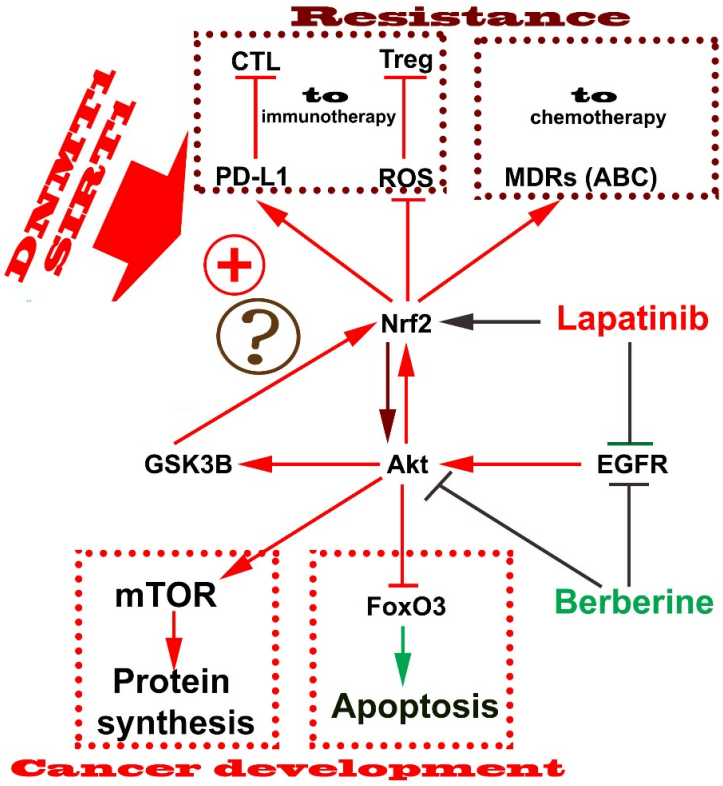

Fig. (2). The key role of Akt/Nrf2 crosstalk and the suggested scheme in our research direction. This scheme shows Akt plays its role as the master driver in cancer development and resistance to therapy. Akt/Nrf2 crosstalk has the responsibility in this regard. The Akt and Nrf2 also modulate anticancer immunity by promoting regulatory T cells as well as PD-L1 upregulation. DNMT1 and SIRT1 are two epigenetic regulators that may control Akt and Nrf2 activities. Red arrows: the processes involved in cancer development and drug resistance; green arrow: the process involved in anticancer activities. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

mutations in several loci including the Akt locus at 14q32.33 (www.ensembl.org) and EGFR-independent activation of Akt.

The EGFR promotes cell survival and proliferation through the activation of PI3K/Akt signaling and KRAS/MAP kinase pathways. Previous findings have shown the activation of AXL, the epithelial-to-mesenchymal (EMT) – associated receptor, in TNBC, activates Akt in EGFR- independent manner [6]. Accordingly, suppression of AXL enhances EGFR-TKI cytotoxicity in MDA-MB-231 cells [7]. EGFR-independent activation of Akt by FAM83A may be another reason for resistance to anti-EGFR therapy in MDA-MB-231 cells [8]. The Akt has also crosstalk with the wnt/β-catenin pathway and accordingly, induces EMT and cancer stem cell growth [9].

The effects of lapatinib on Akt have been previously shown in TNBC cells, including the MDA-MB-231 cell line. It has been shown that p-Akt was reduced in TNBC cells including MDA-MB-231 when lapatinib concentration increased up to 10 μM [10]; however, higher concentrations of lapatinib (up to 50 μM) applied in our research showed a reversed effect on p-Akt. Although lapatinib has been used in much higher concentrations than 50μM in some preclinical studies [11], higher concentrations of lapatinib endanger patients' life, and instead, it is administered in combination with another drug in clinical studies [12, 13]. On the other hand, the mutated-Her2 expression has been reported in MDA-MB-231 cells which may change the behavior of this cell line to lapatinib. The overexpression of nuclear factor erythroid 2-related factor (Nrf2), a key driver in anti-oxidant signaling, is also reported in lapatinib-resistant MDA-MB-231 cells [14]. The Nrf2 is also activated by Akt through which several anti-oxidant genes are expressed [15]. Nrf2 reduces reactive oxygen species (ROS) and therefore, inhibits ROS-dependent apoptosis [16]. Nrf2 has also been reported in MDA-MB-231 cells regulated by Akt, which determines the fate of cancer cells [17]. It expresses multidrug resistance (MDR) genes as well [18]. Therefore, gene expressions, lapatinib concentration, and laboratory conditions may affect cellular response to lapatinib. In contrast, we found that berberine has inhibitory effects on p-Akt in different concentrations (12.5-50 μM). Similarly, another study reported berberine reversed resistance to lapatinib in MDA-MB-231 through Nrf2 inhibition [19]. It has also been shown Akt and Nrf2 have crosstalk in MDA-MB-231 cells and can affect each other [17]. It was previously reported mutated KRAS inhibited EGFR, and MAPK pathway was therefore, triggered in an EGFR-independent manner [20]. In addition, we found that KRAS was upregulated in 10 TNBC cell lines, including MDA-MB-231. Therefore, MDA-MB-231 cells have EGFR-independent upregulation of KRAS/MAPK signaling with a baseline level of PI3K/Akt pathway. We also found Nrf2 is upregulated in MDA-MB-231 cells (unpublished data). Our data showed lapatinib enhanced PI3K/Akt signaling as an alternative pathway to ensure the viability of MDA-MB-231 cells. We thus speculate that lapatinib promotes Akt via Nrf2 activation or vice versa as a cellular defense of MDA-MB-231 cells against lapatinib treatment (Fig. **2**).

To sum up, multi-kinase inhibitors, such as berberine, and more specifically, Akt inhibitors can overcome lapatinib resistance in TNBC cells. Therefore, our finding suggested Akt as a crossroads for resistance to EGFR/Her2-targeted therapy. Our future research will focus on the mechanism of epigenetic regulation on the Akt/Nrf2 axis in detail to discover drugs against epigenetic regulators. We hope to show drugs against HDACs and/or DNMTs in combination with Akt inhibitors are more effective than the use of EGFR/Her2-targeted therapy alone in treating TNBC.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Beretta, G.L. The Molecular Oncology of Drug Resistance: Targets, Drugs and Chemical Biology. *Curr. Med. Chem.,* **2019**, *26*(33), 6018-6019. http://dx.doi.org/10.2174/0929867326666190913194602 PMID: 31518215

[2] Tang, K.D.; Ling, M-T. Targeting drug-resistant prostate cancer with dual PI3K/mTOR inhibition. *Curr. Med. Chem.,* **2014**, *21*(26), 3048-3056. http://dx.doi.org/10.2174/0929867321666140414100127 PMID: 24735368

[3] Liu, Z.; He, K.; Ma, Q.; Yu, Q.; Liu, C.; Ndege, I.; Wang, X.; Yu, Z. Autophagy inhibitor facilitates gefitinib sensitivity in vitro and in vivo by activating mitochondrial apoptosis in triple negative breast cancer. *PLoS One,* **2017**, *12*(5)e0177694 http://dx.doi.org/10.1371/journal.pone.0177694 PMID: 28531218

[4] Rosell, R.; Karachaliou, N.; Morales-Espinosa, D.; Costa, C.; Molina, M.A.; Sansano, I.; Gasco, A.; Viteri, S.; Massuti, B.; Wei, J.; González Cao, M.; Martínez Bueno, A. Adaptive resistance to targeted therapies in cancer. *Transl. Lung Cancer Res.,* **2013**, *2*(3), 152-159. http://dx.doi.org/10.3978/j.issn.2218-6751.2012.12.08 PMID: 25806228

[5] Jabbarzadeh Kaboli, P.; Leong, M.P-Y.; Ismail, P.; Ling, K-H. Antitumor effects of berberine against EGFR, ERK1/2, P38 and AKT in MDA-MB231 and MCF-7 breast cancer cells using molecular modelling and in vitro study. *Pharmacol. Rep.,* **2019**, *71*(1), 13-23. http://dx.doi.org/10.1016/j.pharep.2018.07.005 PMID: 30343043

[6] Meyer, A.S.; Miller, M.A.; Gertler, F.B.; Lauffenburger, D.A. The receptor AXL diversifies EGFR signaling and limits the response to EGFR-targeted inhibitors in triple-negative breast cancer cells. *Sci. Signal.,* **2013**, *6*(287), ra66. http://dx.doi.org/10.1126/scisignal.2004155 PMID: 23921085

[7] Su, C.M.; Chang, T.Y.; Hsu, H.P.; Lai, H.H.; Li, J.N.; Lyu, Y.J.; Kuo, K.T.; Huang, M.T.; Su, J.L.; Chen, P.S. A novel application of E1A in combination therapy with EGFR-TKI treatment in breast cancer. *Oncotarget,* **2016**, *7*(39), 63924-63936. http://dx.doi.org/10.18632/oncotarget.11737 PMID: 27590506

[8] Lee, S.Y.; Meier, R.; Furuta, S.; Lenburg, M.E.; Kenny, P.A.; Xu, R.; Bissell, M.J. FAM83A confers EGFR-TKI resistance in breast cancer cells and in mice. *J. Clin. Invest.,* **2012**, *122*(9), 3211-3220. http://dx.doi.org/10.1172/JCI60498 PMID: 22886303

[9] Dwivedi, A.R.; Thakur, A.; Kumar, V.; Skvortsova, I.; Kumar, V. Targeting Cancer Stem Cells Pathways for the Effective Treatment of Cancer. *Curr. Drug Targets,* **2019**, *20*, 1-21. http://dx.doi.org/10.2174/1389450120666190821160730 PMID: 31433755

[10] Liu, C-Y.; Hu, M-H.; Hsu, C-J.; Huang, C-T.; Wang, D-S.; Tsai, W-C.; Chen, Y-T.; Lee, C-H.; Chu, P-Y.; Hsu, C-C.; Chen, M.H.; Shiau, C.W.; Tseng, L.M.; Chen, K.F. Lapatinib inhibits CIP2A/PP2A/p-Akt signaling and induces apoptosis in triple negative breast cancer cells. *Oncotarget,* **2016**, *7*(8), 9135-9149. http://dx.doi.org/10.18632/oncotarget.7035 PMID: 26824320

[11] Spector, N.L.; Robertson, F.C.; Bacus, S.; Blackwell, K.; Smith, D.A.; Glenn, K.; Cartee, L.; Harris, J.; Kimbrough, C.L.; Gittelman, M.; Avisar, E.; Beitsch, P.; Koch, K.M. Lapatinib Plasma and Tumor Concentrations and Effects on HER Receptor Phosphorylation in Tumor. *PLoS One,* **2015**, *10*(11), e0142845-e0142845. http://dx.doi.org/10.1371/journal.pone.0142845 PMID: 26571496

[12] Yardley, D.A.; Hart, L.L.; Ward, P.J.; Wright, G.L.; Shastry, M.; Finney, L.; DeBusk, L.M.; Hainsworth, J.D. Cabazitaxel Plus Lapatinib as Therapy for $HER2^+$ Metastatic Breast Cancer With Intracranial Metastases: Results of a Dose-finding Study. *Clin. Breast Cancer,* **2018**, *18*(5), e781-e787. http://dx.doi.org/10.1016/j.clbc.2018.03.004 PMID: 29678476

[13] Wisinski, K.B.; Tevaarwerk, A.J.; Burkard, M.E.; Rampurwala, M.; Eickhoff, J.; Bell, M.C.; Kolesar, J.M.; Flynn, C.; Liu, G.; Phase, I. Phase I Study of an AKT Inhibitor (MK-2206) Combined with Lapatinib in Adult Solid Tumors Followed by Dose Expansion in Advanced HER2+ Breast Cancer. *Clin. Cancer Res.,* **2016**, *22*(11), 2659-2667. http://dx.doi.org/10.1158/1078-0432.CCR-15-2365 PMID: 27026198

[14] Ebelt, N.D.; Kaoud, T.S.; Edupuganti, R.; Van Ravenstein, S.; Dalby, K.N.; Van Den Berg, C.L. A c-Jun N-terminal kinase inhibitor, JNK-IN-8, sensitizes triple negative breast cancer cells to lapatinib. *Oncotarget,* **2017**, *8*(62), 104894-104912. http://dx.doi.org/10.18632/oncotarget.20581 PMID: 29285221

[15] Zhang, H-S.; Du, G-Y.; Zhang, Z-G.; Zhou, Z.; Sun, H-L.; Yu, X-Y.; Shi, Y-T.; Xiong, D-N.; Li, H.; Huang, Y-H. NRF2 facilitates breast cancer cell growth via HIF1□-mediated metabolic reprogramming. *Int. J. Biochem. Cell Biol.,* **2018**, *95*, 85-92. http://dx.doi.org/10.1016/j.biocel.2017.12.016 PMID: 29275212

[16] Seo, S.U.; Kim, T.H.; Kim, D.E.; Min, K-J.; Kwon, T.K. NOX4-mediated ROS production induces apoptotic cell death via down-regulation of c-FLIP and Mcl-1 expression in combined treatment with thioridazine and curcumin. *Redox Biol.,* **2017**, *13*, 608-622. http://dx.doi.org/10.1016/j.redox.2017.07.017 PMID: 28806703

[17] De Blasio, A.; Di Fiore, R.; Pratelli, G.; Drago-Ferrante, R.; Saliba, C.; Baldacchino, S.; Grech, G.; Scerri, C.; Vento, R.; Tesoriere, G. A loop involving NRF2, miR-29b-1-5p and AKT, regulates cell fate of MDA-MB-231 triple-negative breast cancer cells. *J. Cell. Physiol.,* **2020**, *235*(2), 629-637. http://dx.doi.org/10.1002/jcp.29062 PMID: 31313842

[18] Cen, J.; Zhang, L.; Liu, F.; Zhang, F.; Ji, B-S. Long-Term Alteration of Reactive Oxygen Species Led to Multidrug Resistance in MCF-7 Cells. *Oxid. Med. Cell. Longev.,* **2016**, *2016*7053451 http://dx.doi.org/10.1155/2016/7053451 PMID: 28058088

[19] Zhang, R.; Qiao, H.; Chen, S.; Chen, X.; Dou, K.; Wei, L.; Zhang, J. Berberine reverses lapatinib resistance of HER2-positive breast cancer cells by increasing the level of ROS. *Cancer Biol. Ther.,* **2016**, *17*(9), 925-934. http://dx.doi.org/10.1080/15384047.2016.1210728 PMID: 27416292

[20] Young, A.; Lou, D.; McCormick, F. Oncogenic and wild-type Ras play divergent roles in the regulation of mitogen-activated protein kinase signaling. *Cancer Discov.,* **2013**, *3*(1), 112-123. http://dx.doi.org/10.1158/2159-8290.CD-12-0231 PMID: 23103856