Endocrine therapy resistance and epithelial to mesenchymal transition are driven by Nicastrin and Notch4 cooperation in MCF7 breast cancer cells

Monica Faronato, Ylenia Lombardo, R Charles Coombes

Imperial College London, Division of Surgery and Cancer, Department of Oncology, Hammersmith Hospital Campus, Du Cane Road, London, W12 0NN, UK

Endocrine therapy resistant (ETR) tumors often display mesenchymal features, associated with aggressive and enhanced motility behaviour. Notch signalling is over-activated in ETR cells. By blocking it, it is possible to interfere with the cell growth. Notch is also implicated in regulating epithelial to mesenchymal transition (EMT) affecting both migration and invasion in breast cancer cells. We know that Nicastrin is the major component of the multi-subunit protease complex Gamma Secretase (GS) which executes intramembrane proteolysis of integral proteins such Notch. We used two models of Endocrine Therapy Resistant MCF7 breast cancer cell lines which acquired EMT feature and showed higher levels of Nicastrin and Notch targets. Moreover, they displayed higher Notch4 levels but lower Notch1 and Notch2, indicating a possible switch of the Notch signalling when cells acquire resistance. We demonstrated that Gamma Secretase inhibitor PF03084014 and anti-NicastrinMAbs were particularly effective in partially inhibiting the EMT process by blocking Nicastrin-Notch4 contribution to resistance. Importantly, we also demonstrated that the stem cells-like population was reduced upon these treatments. Both Nicastrin and Notch4 genetic silencing lead to similar effects. Finally, stably overexpressing Nicastrin, was sufficient to activate Notch4 in MCF7 and render them unresponsive to tamoxifen. Here we highlight our recent study.

Keywords: Breast Cancer; Endocrine Therapy Resistance; Notch4, Nicastrin; Monoclonal Antibodies; ERα; Gamma Secretase Inhibitors

To cite this article: Monica Faronato, et al. Endocrine therapy resistance and epithelial to mesenchymal transition are driven by Nicastrin and Notch4 cooperation in MCF7 breast cancer cells. Can Cell Microviron 2014; 1: e356. doi: 10.14800/ccm.356.

Copyright: © 2014 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

Introduction

Estrogen Receptor (ER) is essential for development and growth of breast cancer (reviewed in [1]). ER+ breast cancers benefit from endocrine therapy (ET) to block ER signaling. Alas, the therapy success is often shadowed by common acquired and intrinsic resistance [2]. It is known that 4-hydroxytamoxifen (4-OH Tam) resistant cells can display an aggressive phenotype developing EMT, and
acquiring a metastatic capacity [3]. Recently, a wide proteomic analysis was conducted on MCF7 cells cultured under 4-OH Tam for a limited period of time (12 months). It revealed that those cells adapted to the drug pressure by the activation of different survival pathways, including ER signaling down-regulation and enhanced motility [4]. In fact, cells that develop 4-OH Tam resistance can escape death by activating alternative pathways. Indeed, epidermal growth factor receptor (EGFR) and human epidermal growth factor 2 (HER2) are involved in cells re-growth [5, 6]. It is of recent understanding that the Notch signaling is amplified in ETR breast cancer cells due to epigenome reprogramming. By inhibiting Notch signaling, the resistant cells growth cells growth is abrogated. More importantly, Notch can be used to discriminate between patients that could fail to respond to therapy [7]. Rizzo and Colleagues demonstrated that Notch and ER cooperate in therapy resistant cells. In more details, estradiol can affect Notch cellular distribution and this can be reverted by Tamoxifen treatment reactivating Notch and proving Notch is required to Breast Cancer cells [8]. Besides, ERα target genes can be activated via IKKα-dependent mechanism through Notch activation suggesting a Notch-ERα crosstalk [9]. The use of Gamma Secretase inhibitors to inhibit Notch in combination with tamoxifen has been proven efficient in ERα+ breast cancer in vivo models [8, 9].

There is a growing body of evidence linking Notch signaling to epithelial to mesenchymal transition (EMT) and cancer progression. [10] There are data showing that breast cancer could arise from a subpopulation of mammary stem/progenitor cells, responsible for origination and maintenance of cancer [11](and reviewed in [12]). Notch pathway was found implicated in controlling the population of breast cancer and breast cancer stem cells [13, 14]. Harrison and colleagues have showed that breast cancer stem cells (BCSC) activity depends on Notch4 receptor whose levels are higher in these cells. Abrogation of Notch4 had indeed the greater effect on BCSC activity in vivo as well as in vitro reducing mammosphere formation [15]. Our group already showed that Nicastrin (NCT), the extracellular component of the GS complex, is highly expressed in breast cancers and confers worst overall survival in ERα-ve tumours [16]. Importantly, we also showed that Nicastrin is important for BCSC population expansion and their invasive features [11]. Nicastrin has an extracellular domain which makes it a potential target for monoclonal antibody therapy (mAb). Haywashi and colleagues synthesized a monoclonal antibody against the extracellular domain of Nicastrin reporting the neutralisation of GS activity. We fully characterized two mAbs showing tumor reduction in vivo of triple negative breast cancer cell lines [16] and [17]. In this paper, we highlight our recent study [18] on the efficacy of GSI PF03084014 and anti-Nicastrin mAbs to partially revert the EMT process and re-sensitize ETR breast cancer cells. We used two models of ETR: MCF7 derived cells treated with 4-OH-Tam for one year and long-term estrogen-deprived cells (LTED), which have gradually acquired resistance after culture in estrogen/steroid-free conditions, modelling aromatase inhibitor resistance. We confirmed that the cells acquire EMT features and become highly invasive and migratory. We showed how these cells express high levels of Nicastrin and Notch4 and concomitantly low levels of Notch1 and Notch2. This would suggest a possible switch after acquiring endocrine resistance. Targeting Notch4 and Nicastrin via genomic silencing, GSI PF03084014, and two mAb directed against Nicastrin, profoundly affected the invasive and migratory capacity of these cells. In fact, we were able to reduce both migration and invasion of 50%. We confirmed majority of EMT genes were significantly reduced and so the main Notch targets. We analyzed E-Cadherin expression by immunofluorescence and showed how after Nicastrin and Notch4 siRNA, and mAbs treatment, E-cadherin re-localized to cell-cell junction. Furthermore, we assessed the cancer stem cells population in TAM-R cells demonstrating a higher content of CD44+/CD24- compared to parental. Following mAbs and GSI treatment, this number was significantly reduced. Importantly, when we fractionated the stem cells population, and compared TAM-R cells with parental, we revealed Nicastrin was highly expressed in the membrane fraction, whereas Notch4 was higher both in the membrane and in the nucleus. This left us speculating an important role of these proteins in the putative ETR population.

Finally, to additionally test Nicastrin contribution in ETR models, we decided to make a Nicastrin stably transfected MCF7. Nicastrin over-expression induced Notch1, 2, and 4 activation. Likewise, Notch targets levels were increased. Nicastrin over-expressing cells showed increased EMT genes expression and promoted the migratory and invasive capacity of the cells. Importantly, Nicastrin overexpression was sufficient to make the cells unresponsive to Tamoxifen. Finally, Notch4 depletion was sufficient to reduce Nicastrin levels and more importantly we re-stored the Tamoxifen response. We conclude Notch4 and Nicastrin cooperate in ETR cells through a positive feedback suggesting a direct role of Notch4 in endocrine resistance Nicastrin mediated.

**Summary**

At least 40% of Breast Cancer patients will develop resistance to endocrine therapy. A growing body of evidence suggests that Notch signaling contributes to endocrine therapy resistance. Recently, we reported that Notch4 and Nicastrin cooperate in developing resistance in MCF7 breast cancer cell lines. We showed that endocrine resistant cells have increased migratory and invasive capacity. Importantly, we demonstrated a Notch1 to
Notch4 switch. Blocking Nicastrin and Notch4 signaling using siRNA treatment was sufficient to attenuate invasion and migration. These results were mirrored by GSI and mAbs against Nicastrin resulting into a partial MET. Finally, Nicastrin stable over-expressing MCF7 was sufficient to bypass tamoxifen response and Notch4 inhibition was sufficient to restore tamoxifen sensitivity.

**Conflicting interests**

The authors have declared that no competing interests exist.

**Acknowledgments**

We thank Luca Magnani, Valentina Vircillo, and Aleksandra Filipovic for their assistance with this project.

**References**