Going off the grid: ERα breast cancer beyond estradiol

Ylenia Perone* and Luca Magnani*

Department of Surgery and Cancer, Imperial Centre for Translational and Experimental Medicine,
Imperial College London, London, UK
*(Y Perone and L Magnani contributed equally to this work)

Correspondence
should be addressed
to L Magnani
Email
l.magnani@imperial.ac.uk

Abstract

Novel studies have linked cholesterol biosynthesis to drug resistance in luminal breast cancer. Structural data suggest that cholesterol metabolites, including 27-hydroxycholesterol (27HC), can act as ERα ligands in these cells. Additionally, hypercholesterolemia has now been linked to breast cancer progression. The focus of this review is to briefly summarize these recent findings and discuss how epigenetic reprogramming is definitively connected to endogenous cholesterol biosynthesis. We elaborate on how these data support a working model in which cholesterol biosynthesis promotes autocrine, pro-invasive signaling via activation of a series of closely related transcription factors. Importantly, we discuss how this mechanism of resistance is specifically associated with aromatase inhibitors. Finally, we examine how the field is now considering the development of anticholesterol therapeutics and companion biomarkers to stratify and treat ERα breast cancer patients. In particular, we review recent progress in pharmaceutical strategies targeting the cholesterol molecular machinery in primary and secondary breast cancers.

Breast cancer is the most common cancer in women and, despite significant progress in detection and treatments, incidence has been steadily increasing in the last 40 years (Torre *et al.* 2015). Over the past decades, it has become clear that breast cancer is a heterogeneous disease characterized by many distinct molecular subtypes. The largest subgroup of breast cancer patients is found in the luminal subtype, representing around 70% of all breast cancer cases. These tumors are positive for estrogen receptor α (ERα), a nuclear-receptor transcription factor promoting cell proliferation and tumor growth. Multiple drugs, commonly referred to as endocrine therapies, have been developed to inhibit ERα activation, representing one of the first examples of targeted therapy. Endocrine therapies can reduce mortality and relapse rate up to 50% (Early Breast Cancer Trialists' Collaborative Group *et al.* 2011).

Although endocrine therapies target the same nuclear receptor (NR), they are characterized by different mechanisms of action. For example, selective estrogen receptor modulators, such as tamoxifen, antagonize the receptor in breast tissue but can have agonistic effect in the endometrium. Aromatase inhibitors (AI), such as letrozole, anastrozole and exemestane, are used primarily in postmenopausal women and block peripheral aromatase activity, thus lowering circulating estrogen levels. Finally, Faslodex/Fulvestrant is a selective estrogen receptor downregulator commonly used in the metastatic breast cancer setting. Although these drugs are particularly effective, over 30% of these patients eventually develop disease recurrence, often due to endocrine therapy resistance (Early Breast Cancer Trialists' Collaborative Group *et al.* 2011). Resistance to endocrine therapies is multifactorial and involves several molecular

events. However, it was not clear if the specific type of treatment would play a significant role in this process. In a recent study, we have characterized, for the first time, the net effect of each treatment with a particular focus on the epigenetic changes occurring in response to each therapy (Nguyen *et al.* 2015). Using epigenomic, genome-wide analyses, we have uncovered how cells developing resistance to AI endogenously trigger cholesterol biosynthesis leading to sustained estrogen-independent ERα activation (Nguyen *et al.* 2015). The link between hypercholesterolemia and breast cancer was previously known since obesity and metabolic disease are two of the strongest risk factors for this tumor development. In particular, recent evidence suggest that endogenous oxysterols including 27-hydroxycholesterol (27HC) link hypercholesterolemia and breast cancer pathophysiology (Nelson *et al.* 2013, Wu *et al.* 2013) (Fig. 1).

Our work demonstrates that cholesterol biosynthesis is activated by changing the chromatin environment around potential SREBP1 (sterol response element binding protein 1) binding sites (Nguyen *et al.* 2015). This was revealed by profiling AI-resistant breast cancer cells using DNAseI hypersensitivity assays coupled with next-generation sequencing. In response to intracellular cues, SREBP1 translocates from endoplasmic reticulum to Golgi and from here to the nucleus activating cholesterol genes by binding to their promoters (i.e. hydroxy-methyl-glutaryl CoA reductase (HMGCR) and squalene epoxidase (*SQLE*)). Recently, a new series of compounds (fatostatins) have been designed to block SREBP1 nuclear translocation (Kamisuki *et al.* 2009). Treating AI-resistant cells with fatostatins was sufficient to reduce ERα and block cell invasion in AI-resistant cells (Nguyen *et al.* 2015). Strikingly, these data indicate that epigenetic reprogramming might induce new transcription factor dependencies including SREBP1/2. Before formulating therapeutic strategies based on epigenetic modulators, several basic questions need to be addressed. How is SREBP1 activated in endocrine therapy-resistant breast cancer? At what stage is this activation required? Is CB only acting through ERα or are
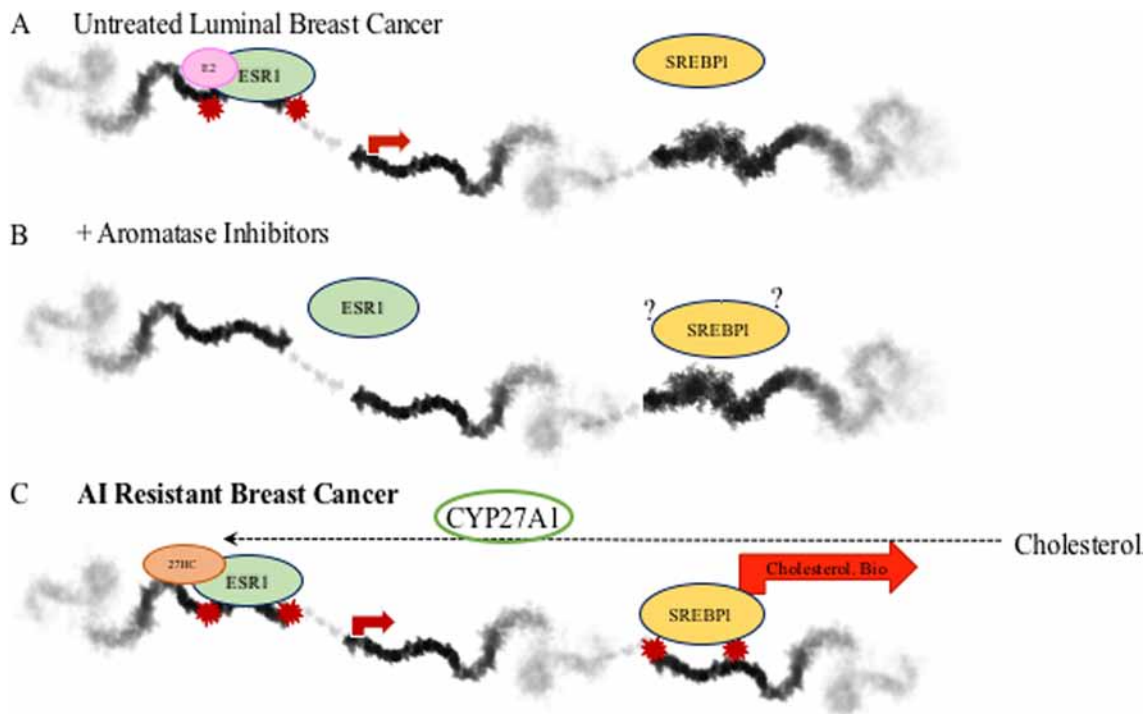

Figure 1
Schematic model of aromatase inhibitors-resistant ERα breast cancer. (A) ERα breast cancer proliferates in response to circulating estrogens. ERα activates gene transcription (arrow) by binding to epigenetically bookmarked distal enhancers (asterisks). (B) In postmenopausal women, inhibition of androgens peripheral conversion to estrogens is sufficient to impair ERα binding and block ERα-driven transcription. (C) Long-term exposure to aromatase inhibitors can lead to permanent epigenetic reprogramming especially near genes involved in cholesterol biosynthesis. In this scenario, the chromatin structure near these genes become accessible to transcription factors such as SREBP1 leading to endogenous cholesterol biosynthesis. Ultimately, cholesterol is transformed into ERα ligands (i.e. 27HC) and become an alternative fuel for ERα-mediated transcription. DNA is represented in black and gray, red arrow indicates gene transcription. The key molecules involved in this working model are shown in the scheme (estradiol, E2; estrogen receptor 1, ESR1; sterol regulatory binding element 1, SREBP1; 27 hydroxycholesterol, 27HC).

there other receptors involved? Finally, can we exploit this finding in the clinical setting?

Interestingly, 27HC has agonist activity on members of the NR superfamily (Umetani *et al.* 2007) including the liver X receptor (LXR). In the last 40 years, NRs have been widely studied, but, beside ERα, whether other NRs are pivotal in breast cancer is still deeply debated. We know that NRs are activated by several hormones, metabolites, dietary lipids and vitamins, and regulate gene transcription after binding transcription factors (Gadaleta & Magnani 2014). A growing body of evidence shows that not only ERα but also other cholesterol- and oxysterol-activated NRs (CANRs) play a significant role in ERα breast cancer, in particular LXR and estrogen-related receptor-alpha (ERRα). This suggests that anticholesterol therapy in several diseases might be mediated by a larger network of NRs. Our work supports the hypothesis that intratumoral CB is constitutively activated after chronic exposure to AI leading to the local accumulation of metabolic ligands for ERα and possibly for other CANRs such as LXR and FXR (Farnesoid X receptor). Indeed, DNA motifs for LXR and FXR are enriched with AI-resistant super-enhancers (Nguyen *et al.* 2015). Super-enhancers are the key regulatory regions driving cell identity during both development and carcinogenesis (Whyte *et al.* 2013). Active LXR has been characterized as a controversial player. While some studies have reported that LXR activation might be antiproliferative (reviewed in Lin & Gustafsson 2015), others have suggested that it is involved in breast cancer progression (Nelson *et al.* 2013).

CB might also have agonist effects on ERRα activation, another NR-family member (Wei *et al.* 2016). ERRs are a family of NRs homologous to ERα-mediating cholesterol regulation of bone resorption, regulation of fatty acid oxidation, mitochondrial biogenesis, and oxidative phosphorylation, and a modulator of ERα signaling. Interestingly, Wei and coworkers have recently demonstrated that pharmacological regulation by statins in osteoclasts is ERRα dependent. Similarly, we have shown that statins might antagonize breast cancer progression by interfering with ERα activation (Nguyen *et al.* 2015).

Statins might not be the only lipids lowering drugs with potential effects on breast cancer. A recent meta-analysis conducted from the Early Breast Cancer Trialists' Collaborative Group (Early Breast Cancer Trialists' Collaborative Group 2015) displayed the benefits of bisphosphonates treatment in postmenopausal women, reporting significant reductions not only in bone metastasis but also in any distant recurrence site and breast cancer mortality. We know that both lipids lowering drugs and bisphosphonates block osteoclast function and the cholesterol pathway by decreasing the bioavailability of the endogenous ERRα agonist cholesterol (Wei *et al.* 2016). The effects on bone recurrence emphasize the potential importance of blocking CB in highly invasive metastatic breast cancer cells. For example, it might be possible that in addition to bisphosphonates effect on osteoclasts, lowering CB in micrometastases might also antagonize ERα, ERRα and other CANRs. Consequently, disrupting lipid metabolic pathways could induce tumor regression and inhibit metastatic spread. Indeed, several lipids lowering drugs are under preclinical and clinical studies in several cancer types. There are several ways to directly block SREBPs: our group studied fatostatin in breast cancer showing that inhibition of SREBP lowers ERα activation in AI-resistant cells (Nguyen *et al.* 2015). Other drugs, namely sphingomab and nelfinavir, have been designed to block SREBP release and translocation via Site-1 and Site-2 proteases. Furthermore, metformin, an antidiabetic drug, was shown to inhibit breast cancer cell proliferation and the expression of SREBP1-c. On the other hand, statins block HMGCR, while terbinafine is a SQLE inhibitor and bisphosphonates are designed to interfere with farnesyl-PP synthase (as reviewed by Beloribi-Djefaflia *et al.* 2016). HMGCR, SQLE, and farnesyl-PP synthase are three critical enzymes in the CB pathway. Additionally, molecules such as orlistat block fatty acid synthase (FASN), another enzyme of the CB pathway, whereas methyl-b-cyclodextrin modulates lipid raft components to induce cell death signaling. Finally, there are some other molecules under investigation able to modulate NRs such as LXR, or key enzymes like stearoyl-CoA desaturase 1 (SCD1) and cyclooxygenase 2 (COX2) (Beloribi-Djefaflia *et al.* 2016). Overall, the results from our study represent a step toward the AI resistance mechanisms providing evidence that anticancer effects can be potentiated by combining different types of endocrine therapy with strategies depriving the tumor of cholesterol. The rationale for this assumption has its grounds on a study showing that some drugs already on the market for treating ERα breast cancer are also able to block cytochrome P45027A1 (CYP27A1) (Mast *et al.* 2015). This is the only enzyme in humans converting cholesterol to 27HC, the oxysterol-modulating NRs like ERα and LXR. During the menopause, 27HC increases while estrogens decrease suggesting a potential switch in ERα agonists (Umetani *et al.* 2007). In breast cancer, CYP27A1 enzyme expression increases and it correlates with tumor grade (Nelson *et al.* 2013). Mast and coworkers tested several

drugs to target this enzyme and found that anastrozole, for example, not only inhibits CYP19A1, but it is also the strongest inhibitor of CYP27A1. So far, we discussed the development of compounds able to antagonize breast cancer escape mechanisms with endocrine treatment. On the other hand, if our hypothesis is correct, another important potential application of our study could be the translational applicability of using SREBP1 and CB as potential biomarkers to predict AI resistance.

All these pathways converge to a unique scenario where the reprogrammed metabolism plays a key role for growth and proliferation of invasive cancer cells. The first tumor-specific metabolic alteration was described by Warburg that observed proliferating ascites-derived tumor cells convert the majority of glucose to lactate, even in oxygen-rich conditions (aerobic glycolysis) (Warburg 1956). He set the foundations for a traditional model based on metabolic alterations as an indirect response to external signaling. Recently, a reverse Warburg has been proposed (Pavlides *et al.* 2009), in which the stroma can provide metabolites useful for cancer cells. Similarly, many infiltrating tumor cells can act as a source of cholesterol derivatives including macrophages and fibroblasts (Nelson *et al.* 2013). These phenomena parallel the astrophysics concept of "Terraforming", based on the hypothetical modifications of a planet environment in order to be similar to the Earth. Similarly, translating this idea at a biological level, cancer cells could change the microenvironment in order to create a more habitable one suggesting that the tumor might "farm" the stroma for CANRs ligands. However, an alternative model might exist. Evidences have been recently gained supporting a supply-based model in which proliferating cells are reprogrammed to meet the challenges of colonizing different environments such as lung, bones, liver or brain (Ward & Thompson 2012). In this model, metabolic reprogramming is crucial to provide endogenous nutrients (in BC namely ligand for CANRs) in order to metastasize. To further support the hypothesis that invasive cells work toward becoming microenvironment independent, a subgroup of reprogrammed cancer cells behave like pioneers becoming invasive and able to colonize and adapt to different microenvironments. In this scenario, CB could operate as a survival toolkit in order for the pioneer metastatic cell to be able to invade a new environment and survive. Indeed, supplying the necessary fuel, we suggested CB playing a key role in drug-resistant relapses making the cell autonomous. Consistent with this hypothesis and in agreement with others, our data showed that epigenetic reprogramming underlies metabolic changes playing a critical role in estrogen-independent cancer cells and promoting self-sustaining signal transduction
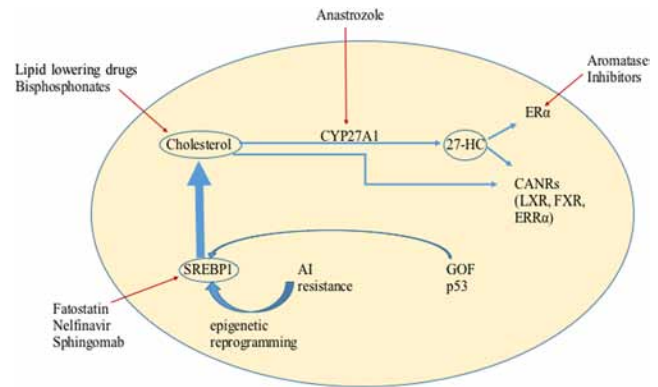

Figure 2

Schematic supply-based model of resistant ERα breast cancer cell. In response to aromatase inhibitors-resistant cancer cells endogenously trigger cholesterol biosynthesis leading to sustained estrogen-independent ERα activation. Metabolic epigenetic reprogramming induce new transcription factor dependencies including SREBP1. Metastatic cells behave like pioneers becoming invasive and able to colonize and adapt to different microenvironments. Cancer cells with *TP53* gain-of-function (GOF p53) are characterized by the upregulation of CB with mutant p53 potentially acting as coactivator for SREBPs proteins. Several drugs are under study in order to block the cholesterol pathway (red arrows).

mechanisms to foster growth and survival (Wu *et al.* 2013, Nguyen *et al.* 2015).

Epigenetic changes promote overexpression of several oncogenic metabolic genes; nonetheless, genetic changes might also contribute to similar metabolic switches. For example, it has been recently shown that breast cancer cells with *TP53* gain-of-function mutations are characterized by the upregulation of CB (Freed-Pastor *et al.* 2012). Indeed, the upregulation of the initial steps of CB is necessary for the effects of mutant p53 on tissue architecture. In addition to being a potential coactivator for SREBP1 proteins, mutant p53 binding enhances SREBPs activation resulting in augmented CB in malignant cells. Mutated p53 binding enhances SREBPs activation resulting in augmented CB in malignant cells. Furthermore, the authors showed that fatostatin significantly reduced the level of mutant p53 binding to the HMGCR gene promoter. Interestingly, CB upregulation significantly correlated with a higher rate of *TP53* mutations in breast cancer patients, and it also correlates with a poor prognosis (Fig. 2).

There are several studies testing lipids lowering drugs anticancer potential, but their protective role in the clinical setting is still greatly debated. For example, a nation-wide study (Ahern *et al.* 2011) found that ERα-positive breast cancer patients who were treated with statins were less likely to develop relapses. Interestingly, pharmacodynamic studies showed no effect of these drugs

on estradiol levels or anastrozole metabolism, laying the first stone at further investigating the possibility of a combined therapy in metastatic breast cancer patients. Clinical trials combining lipid-lowering drugs and AI are needed in order to clarify at a clinical level the new molecular insights that our study highlighted.

Collectively, our data warrant an in-depth investigation toward potential new biomarkers to predict AI resistance *a priori* and to stratify ERα patients to the most appropriate form of therapy while considering lipid-lowering drugs and endocrine combined treatment in breast cancer.

References

Ahern TP, Pedersen L, Tarp M, Cronin-Fenton DP, Garne JP, Silliman RA, Sorensen HT & Lash TL 2011 Statin prescriptions and breast cancer recurrence risk: a Danish nationwide prospective cohort study. *Journal of the National Cancer Institute* **103** 1461–1468. (doi:10.1093/jnci/djr291)

Beloribi-Djefaflia S, Vasseur S & Guillaumond F 2016 Lipid metabolic reprogramming in cancer cells. *Oncogenesis* **5** e189. (doi:10.1038/oncsis.2015.49)

Early Breast Cancer Trialists' Collaborative Group, Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C, *et al.* 2011 Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378** 771–784. (doi:10.1016/S0140-6736(11)60993-8)

Early Breast Cancer Trialists' Collaborative Group 2015 Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* **386** 1341–1352. (doi:10.1016/S0140-6736(15)61074-1)

Freed-Pastor WA, Mizuno H, Zhao X, Langerød A, Moon S-H, Rodriguez-Barrueco R, Barsotti A, Chicas A, Li W, Polotskaia A, *et al.* 2012 Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell* **148** 244–258. (doi:10.1016/j.cell.2011.12.017)

Gadaleta RM & Magnani L 2014 Nuclear receptors and chromatin: an inducible couple. *Journal of Molecular Endocrinology* **52** R137–R149. (doi:10.1530/JME-13-0170)

Kamisuki S, Mao Q, Abu-Elheiga L, Gu Z, Kugimiya A, Kwon Y, Shinohara T, Kawazoe Y, Sato S-I, Asakura K, *et al.* 2009 A small molecule that blocks fat synthesis by inhibiting the activation of SREBP. *Chemistry & Biology* **16** 882–892. (doi:10.1016/j.chembiol.2009.07.007)

Lin C-Y & Gustafsson J-Å 2015 Targeting liver X receptors in cancer therapeutics. *Nature Reviews Cancer* **15** 216–224. (doi:10.1038/nrc3912)

Mast N, Lin JB & Pikuleva IA 2015 Marketed drugs can inhibit cytochrome P450 27A1, a potential new target for breast cancer adjuvant therapy. *Molecular Pharmacology* **88** 428–436. (doi:10.1124/mol.115.099598)

Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, Carver NJ, Pillai RV, Sullivan PM, Sondhi V, *et al.* 2013 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* **342** 1094–1098. (doi:10.1126/science.1241908)

Nguyen VTM, Barozzi I, Faronato M, Lombardo Y, Steel JH, Patel N, Darbre P, Castellano L, Gyorffy B, Woodley L, *et al.* 2015 Differential epigenetic reprogramming in response to specific endocrine therapies promotes cholesterol biosynthesis and cellular invasion. *Nature Communications* **6** 10044. (doi:10.1038/ncomms10044)

Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, Casimiro MC, Wang C, Fortina P, Addya S, *et al.* 2009 The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle* **8** 3984–4001. (doi:10.4161/cc.8.23.10238)

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet Tieulent J & Jemal A 2015 Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians* **65** 87–108. (doi:10.3322/caac.21262)

Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, Korach KS, Shaul PW & Mangelsdorf DJ 2007 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nature Medicine* **13** 1185–1192. (doi:10.1038/nm1641)

Warburg O 1956 On the origin of cancer cells. *Science* **123** 309–314. (doi:10.1126/science.123.3191.309)

Ward PS & Thompson CB 2012 Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell* **21** 297–308. (doi:10.1016/j.ccr.2012.02.014)

Wei W, Schwaid AG, Wang X, Wang X, Chen S, Chu Q, Saghatelian A & Wan Y 2016 Ligand activation of ERRα by cholesterol mediates statin and bisphosphonate effects. *Cell Metabolism* **23** 479–491. (doi:10.1016/j.cmet.2015.12.010)

Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI & Young RA 2013 Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* **153** 307–319. (doi:10.1016/j.cell.2013.03.035)

Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, Thompson B, Girard L, Mineo C, Brekken RA, *et al.* 2013 27-hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Reports* **5** 637–645. (doi:10.1016/j.celrep.2013.10.006)