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Modeling the estrogen receptor to growth factor receptor signaling switch in human breast cancer cells

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ABSTRACT

Breast cancer cells develop resistance to endocrine therapies by shifting between estrogen receptor (ER)-regulated and growth factor receptor (GFR)-regulated survival signaling pathways. To study this switch, we propose a mathematical model of crosstalk between these pathways. The model explains why MCF7 sub-clones transfected with HER2 or EGFR show three GFR-distribution patterns, and why the bimodal distribution pattern can be reversibly modulated by estrogen. The model illustrates how transient overexpression of ER activates GFR signaling and promotes estrogen-independent growth. Understanding this survival-signaling switch can help in the design of future therapies to overcome resistance in breast cancer.

1. Introduction

Mammalian cells can switch between different signaling pathways to achieve distinct physiological goals in response to environmental stimuli, as exemplified by immune cell differentiation [1]. This plasticity is important for normal cells to differentiate properly and to survive in stressful environments. In cancer cells, this plasticity often results in drug resistance including acquired resistance to anti-estrogenic drugs.

The estrogen receptor (ER) and growth factor receptor (GFR) pathways are major drivers of survival and proliferation in 85% of breast tumors [2,3]. In clinical practice, expression of ERα (the most prevalent of two ER genes) and HER2 (a major GFR and member of the EGFR superfamily) are validated biomarkers used to determine treatment strategies for individual patients [4]. Approximately 70% of breast cancers express ERα [5], and various endocrine therapies have been developed to interfere with ER action [5]. Antagonizing GFR pathways (e.g., using trastuzumab) in HER2+ breast cancer also improves disease-free and overall survival for breast cancer patients [6]. However, the ultimate efficacy of therapies targeting individual pathways is not satisfactory. For example, tamoxifen successfully reduces by one-third the annual death rate from breast cancer, but one-third of tamoxifen-treated women develop recurrent disease within 15 years [5]. Resistance to anti-estrogens or GFR pathway antagonists also develops in human breast cancer cell lines [7–9].

We have used mathematical modeling guided by experimental observations to explore the mechanism underlying acquired resistance to endocrine therapies as driven by the ER–GFR switch. Acquired resistance could arise by activation of a compensatory escape pathway when the normal driver pathway is inhibited [3], the so-called 'hybrid-car' model of breast cancer [10]. Since breast cancer cells can switch reversibly and robustly between ER and GFR pathways for proliferation and survival [3,10], blocking either the ER or GFR pathway will usually result in activation of the other, allowing some cells to survive and eventually resume proliferation. Evidence for a close regulatory relationship between ER and GFR

Abbreviations: AKT, a serine/threonine-specific protein kinase, also known as Protein Kinase B (PKB); CCS, charcoal-stripped fetal-calf serum; CSC, cancer stem cell; E2, 17β-estradiol; E2:ER, E2-bound estrogen receptor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ER-P, phosphorylated estrogen receptor; FCS, fetal calf serum; GFR, growth factor receptor; HER2, human epidermal growth factor receptor-2; MAPK, mitogen activated protein kinases; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphatidylinositide 3-kinases

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signaling includes the reciprocal expression of ER and GFR in most breast cancers [11], and activation of GFR pathway components (HER2, EGFR, MAPK, PI3K, AKT, mTOR, NFκB etc.) as compensatory responses to anti-estrogens [5,12–14]. Interestingly, these compensatory processes are reversible after withdrawing the endocrine treatment [15]. Moreover, recent evidence indicates that ER negative (ER−) breast cancer cells may develop resistance to GFR pathway antagonists by restoring the ER pathway and hence becoming responsive to anti-estrogens [16,17].

ER and GFR are sometimes positively associated in breast cancers [18,19]. Whether ER and GFR are negatively or positively correlated depends on how ER is activated. ER can be activated either by binding to 17β-estradiol (E2, the primary estrogen present in breast tumors) to form an active E2:ER complex, or by phosphorylation (ER-P) by various kinases (e.g., ERK and AKT) at multiple sites [5,20,21]. E2:ER has an inhibitory effect on GFR. E2 withdrawal can release the inhibition of ER on GFR expression and NFκB activity [22–26], consistent with the fact that E2:ER binds the promoter region of GFR genes (e.g., HER2 and EGFR) and acts as a repressor [27,28]. However, E2-independent ER-P is positively associated with GFR, and it can up-regulate certain ligands (e.g., TGFα, EGF and amphiregulin) of the GFR signaling network, which in turn activate the kinases that phosphorylate more ER [29–31]. This auto-activation loop has been implicated in tamoxifen-resistance [31,32]. NFκB, a major integrator of the GFR signaling network, is involved with E2:ER in a mutual-inhibition feedback loop [24,33]. NFκB also controls the expression of a broad spectrum of genes regulating important cellular behaviors including cell differentiation [34,35]. In particular, NFκB activates the transcription factor TWIST and represses the expression of E-cadherin, which in turn enhances the epithelial–mesenchymal transition (EMT) in breast cancer [36]. EMT is associated with a de-differentiation process whereby epithelial-like breast cancer cells increase their 'stemness' and undergo a phenotypic transition from HER2− to HER2+ [37]. EMT in breast cancer cells is likely due to genome-scale epigenetic reprogramming, including the promoter activity of HER2 [38]. Epigenetic changes such as methylation or acetylation can occur during differentiation or de-differentiation and are often reversible [36–38].

While the crosstalk between ER and GFR pathways in breast cancer, especially in MCF7 cells, has been widely studied [5,13,20,22,31,39,40], a comprehensive, dynamic view of ER–GFR crosstalk is still lacking. Previously, we proposed a simplified model that could account for the effects of E2 withdrawal on the bimodal distribution of GFR (HER2 or EGFR) in MCF7 cells [41]. However, this model combined all components of the GFR pathway into one variable and required an unreasonably slow rate constant to fit the experimental data. A more realistic model would allow the GFR pathway to exhibit both rapid (e.g., post-translational modifications of GFR proteins) and slow modifications (e.g., epigenetic modifications of GFR promoters). Moreover, a recent report indicates that transient ER overexpression can robustly activate E2-independent growth of MCF7 cells [42], suggesting further modifications to achieve a more realistic model.

Here we present a new model to explore the mathematical characteristics of the ER–GFR switch that is a central determinant of breast cancer cell fate in response to endocrine therapies. The model explains many aspects of the available experimental data (Supplementary document, Fig. S1–S4), for example: (1) in sub-clones of MCF7 cells transfected with GFR (HER2 or EGFR), there are three different distribution patterns of GFR [43,44] (Fig. S1), (2) for sub-clones with a bimodal distribution of GFR, the distribution can be reversibly manipulated by varying E2 levels [43,44] (Fig. S2), (3) whereas E2 withdrawal in GFR-transfected MCF7 cells switches on GFR expression within weeks, E2 addition takes months to switch off expression [43,44] (Fig. S2), (4) E2 withdrawal can up-regulate GFR expression within 5 weeks in GFR-transfected MCF7 cells, but fails to do so in wild type MCF7 cells [43,44] (Fig. S3), and (5) transient ER overexpression in MCF7 cells can switch on the GFR pathway and promote E2-independent growth [42] (Fig. S4). The model provides a new tool to understand and evaluate these intriguing experimental observations, and it may help in finding new strategies to overcome anti-estrogen resistance in breast cancer.

2. Materials and methods

We postulate a highly condensed model of the interaction between ER and GFR (Fig. 1A and Supplementary documents). The protein level of GFR is down-regulated by E2:ER complex [27,28]. After E2 withdrawal, GFR is released from inhibition and its downstream kinases phosphorylate ER to an E2-independent form, ER-P [5,20,21]. ER-P can activate and stabilize the GFR pathway, creating a positive feedback loop [29–31]. In addition, GFR further activates transcription factors such as NFκB, promoting a series of epigenetic changes contributing to increased GFR expression and establishing another positive feedback loop [34,35]. For simplicity, we combine the epigenetic factors contributing to GFR expression into the quantity '*EPI*'. '*E2ER*' and '*ERP*' are used to represent [E2:ER] and [ER-P]. The wiring diagram in Fig. 1A was translated into ordinary differential equations (ODEs) by a formalism that allows us to capture complex dependencies in a simple manner [45] for simulation and analysis. We used the program XPP-AUT, available freely at http://www.math.pitt.edu/~bard/xpp/xpp.html, to simulate the model and to draw bifurcation diagrams. The ensemble stochastic simulations were performed with Matlab Version 7.9.0. A detailed version of materials and methods is provided in the Supplementary document.

3. Results

3.1. Bifurcation analysis of the survival-signaling switch

The nullclines of our system of equations (Eq. S1 and S2) are plotted in Fig. 1B. The intersections of these two curves correspond to steady states of the model. The number of steady states is controlled by the value of *E2* level. When $E2 = 1$, there is one stable steady state corresponding to low *GFR* and low *EPI* (GFR−/EPI−). When *E2* is reduced below 0.65, there are three steady states, two of which are stable and a third which is unstable. The stable steady states have GFR and EPI levels that are either both low (GFR−/EPI−) or both high (GFR+/EPI+). Fig. 1C illustrates how the steady states of the system change with *E2*. The system has three steady states in the range of $0 < E2 < 0.65$ and only one stable steady state when $E2 > 0.65$. However, *E2* is not the only parameter that influences the system's bistability. *GFRover*, which represents the influence of additional GFR genes transfected into MCF7 cells, can also be used as a bifurcation parameter. Fig. 2A shows that when *E2* is held constant at $E2 = 1$ the system is bistable only when $3.2 < GFRover < 12.8$. We will show how this bistable survival-signaling switch can explain the results of several important experiments that are difficult to understand without a model.

3.2. Three distribution patterns of GFR

Liu et al. transfected HER2 cDNA into MCF7 cells and created multiple stable sub-clones, which were further screened for HER2 protein expression levels using flow cytometry. Interestingly, three HER2 distribution patterns were observed in the sub-clones they selected [43]: (1) a single peak of cells with elevated HER2 protein (MB4 in Fig. 2B), (2) a single peak of cells with low HER2