Computational Biology and Chemistry 59 (2015) 42–54

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research Article

Crosstalk events in the estrogen signaling pathway may affect tamoxifen efficacy in breast cancer molecular subtypes

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ARTICLE INFO

Article history:
Received 24 February 2015
Received in revised form 2 July 2015
Accepted 10 July 2015
Available online 19 August 2015

Keywords:
Pathway crosstalk
Breast cancer
Estrogen signaling
Systems biology

ABSTRACT

Steroid hormones are involved on cell growth, development and differentiation. Such effects are often mediated by steroid receptors. One paradigmatic example of this coupling is the estrogen signaling pathway. Its dysregulation is involved in most tumors of the mammary gland. It is thus an important pharmacological target in breast cancer. This pathway, however, crosstalks with several other molecular pathways, a fact that may have consequences for the effectiveness of hormone modulating drug therapies, such as tamoxifen. For this work, we performed a systematic analysis of the major routes involved in crosstalk phenomena with the estrogen pathway – based on gene expression experiments (819 samples) and pathway analysis (493 samples) – for biopsy-captured tissue and contrasted in two independent datasets with *in vivo* and *in vitro* pharmacological stimulation. Our results confirm the presence of a number of crosstalk events across the estrogen signaling pathway with others that are dysregulated in different molecular subtypes of breast cancer. These may be involved in proliferation, invasiveness and apoptosis-evasion in patients. The results presented may open the way to new designs of adjuvant and neoadjuvant therapies for breast cancer treatment.

1. Introduction

Breast cancer is a complex disease (Polyak, 2011) with a very heterogeneous nature; several attempts at classifying the disease have been developed, at the clinical (Viale, 2012), histopathological (Malhotra et al., 2010), and molecular level (Sørlie et al., 2001). Said differences give way to different therapeutical approaches to deal with different prognosis.

Mammary epithelial cells can be stimulated by estrogen, acting as a survival and proliferation signal (Levin, 2005). Canonically, estrogen binds to nuclear receptors, acting as a transcription factor. Estrogen receptors are also found on the membrane, which can activate second messenger proteins to relay the estrogen signal and exert physiological changes. These second messenger proteins can also directly affect estrogen receptors, modifying their affinity for ligands and for DNA and therefore changing their transcription factor activity. These interactions constitute the *Estrogen Signaling Pathway* (ESP), a pharmacological target for cancer therapy.

Tamoxifen is a selective estrogen receptor modulator (SERM) used for the neoadjuvant and adjuvant treatment of breast cancer. Its mechanism of action consists in the competitive inhibition of estrogen, inhibiting its various actions at the signaling and transcriptional level.

Adjuvant tamoxifen therapy significantly reduces the risk of recurrence and death from breast cancer in all age groups (Wood et al., 2003; Group et al., 1998). Benefits related to tamoxifen adjuvant therapy include an increase in overall survival and distant disease-free survival, reduction of breast cancer-specific mortality, decreased risk of recurrence, and decreased risk of contralateral breast cancer (Cardoso et al., 2014).

For these reasons, tamoxifen is the election drug in current clinical practice (Burstein et al., 2014; CENETEC, 2009). When compared with cytotoxic chemotherapy, SERMs are well tolerated and are associated with mostly minor toxicities. Despite the relative safety and significant antineoplastic and chemopreventive activities of antiestrogens, most initially responsive breast tumors acquire resistance (Clarke et al., 2003; Viedma-Rodrí guez et al., 2014).

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[1] Guillermo de Anda Jáuregui is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and received fellowship 324432 (420970 // 262974) from CONACYT.

http://dx.doi.org/10.1016/j.compbiolchem.2015.07.004
1476-9271/

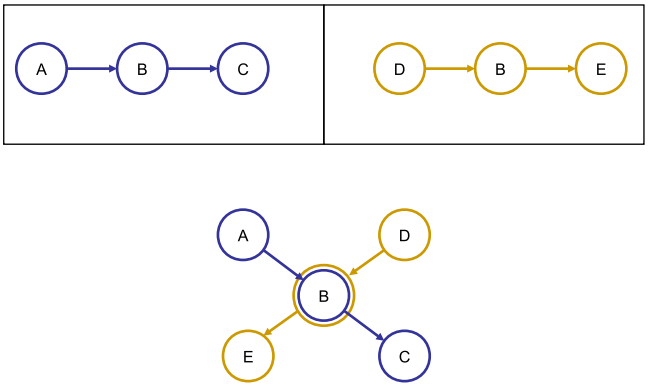

Fig. 1. A schematic view of pathway crosstalk. Pathway **A** → **B** → **C** presents a crosstalk on molecule **B** with the pathway **D** → **B** → **E**.

Breast cancer mortality is a major public health concern, particularly in developing countries. This is partially a result that most cancer cases in low- and middle-income countries are detected at later stages than in high-income countries. For this reason, surgical treatment is generally delayed in these countries, increasing the dependence on pharmacological treatment (Unger-Salda na and Infante-Casta neda, 2009).

In this regard, let us recall that cancer is a pathway-based disease (Hanahan and Weinberg, 2000), and as such, pathway analysis is a powerful tool to understand the complex relationships and interactions that are involved in the pathology. Pathways are useful representations of these interactions, derived from our current knowledge of cellular function. As representations of the molecular biology of the cell, it comes as no surprise that many of these pathways share many molecules, being able to *crosstalk* (see Definition 1 and Fig. 1) with each other (Gilbert, 2000).

These interactions are non-trivial, as many of the processes involved with a physiological (or pathological) function can be working in such a way that an alteration in one pathway leads to an alteration in many others. A *systems biology* approach is thus needed in order to properly contextualize such subtle interactions in a global, integrative manner. By understanding the mechanisms underlying the biological processes, a comprehensive analysis might lead to insights into the molecular phenomenology that further the pathological phenotypes (Hernández-Lemus, 2014).

In this article, we explore deregulation of pathways crosstalking with the ESP, as a probable source of resistance to anti-estrogen therapy.

We propose a pipeline designed to study deregulation of pathways that crosstalk with a pathway of biological interest. For this work, we centered on the Estrogen Signaling Pathway, as it is, as mentioned before, a pharmacological target in the treatment of breast cancer.

We identified the pathways within the KEGG database (Kanehisa and Goto, 2000; Kanehisa et al., 2014) that crosstalk with said the ESP, and the molecules through which said crosstalk occurs.

We studied the enrichment of said pathways in a highly curated dataset consisting in more than 800 microarray samples of breast cancer tumors, from different molecular subtypes.

Given the definition of crosstalk we present, we assume that a pathway's enrichment status may directly be affected by the enrichment status of other pathway, only if this second pathway presents crosstalk (that is, shares at least a molecule) with the first one. We also assume that the deregulation of any one pathway in crosstalk with another pathway may (or may not), be involved in the deregulation of the second.

Definition 1. A **biological network** is formally defined as a graph $\mathcal{G}(V, E)$ over a duplex formed by two sets, a set V of nodes or vertices ($v_i \in V$) given by **biomolecules**, and a set E of edges connecting such vertices ($e_i \in E$) representing **physical** or **chemical** interactions of several classes among such biomolecules. The connectivity rule is represented by the so-called **adjacency matrix** $\mathcal{A} = A_{i,j}$, where $A_{i,j} \neq 0$ implies a non-null interaction between biomolecules v_i and v_j. If the biological network represents a *biochemical* or *metabolic* network, we can define *trajectories* over such network – i.e. specific concatenated sequences of interactions – representing previously defined biological processes. Such trajectories are called **biochemical pathways**.

A given pathway is a trajectory $\mathcal{P}_{m\to n} = v_m \to v_{m+1} \to v_{m+2} \cdots \to v_{n-1} \to v_n$ connecting a series of molecules $v_m, v_{m+1}, v_{m+2}, \ldots, v_{n-1}, v_n$ by interactions leading to a particular biological process. Each one of the successive arrows represent a non-null interaction of strength $A_{m,m+1}$, $A_{m+1,m+2}$, $\ldots A_{n-1,n}$, respectively.

Within this graph approach, we say that **crosstalk** between two pathways $\mathcal{P}_A$ and $\mathcal{P}_B$ occurs if there is a set $\nu^{A,B}$ (containing at least one molecule, $v_i^{A,B}$) such that $\forall v_i^{A,B} \in \nu^{A,B}$ it holds that $v_i^{A,B} \in \mathcal{P}_A$ and $v_i^{A,B} \in \mathcal{P}_B$. If A is the whole set of molecules in $\mathcal{P}_A$ and B the whole set of molecules in $\mathcal{P}_B$ then $\nu^{A,B} = A \bigcap B$. Every molecule $v_i^{A,B} \in \nu^{A,B}$ is said to participate in a **crosstalk event** between pathways $\mathcal{P}_A$ and $\mathcal{P}_B$.

To further explore some of the enriched pathways in relation to tamoxifen resistance, we applied the same methodology to data obtained from experiments in *MCF-7* cell lines with resistance to tamoxifen, as well as data from patients treated with tamoxifen in the adjuvant context.

Finally, we discuss some implications that the deregulation of certain pathways that crosstalk with the ESP (such as *apoptosis* and *immune response* pathways may have in the pharmacological treatment of breast cancer.

2. Materials and methods

2.1. Analysis pipeline

Our work proposes an analysis pipeline described in Fig. 2. This pipeline takes data from current biological knowledge (pathway databases) and experimental expression data of different phenotypes. Pathways that crosstalk with a pathway of biological interest are identified, and Pathway enrichment profiles are generated for each of the phenotypes studied. This information is then used to generate biological hypothesis to be tested.

In our work, we take pathways from the KEGG database and identify those that crosstalk with our pathway of biological interest, the *Estrogen Signaling Pathway*. Expression data of breast cancer samples classified into molecular subtypes is used. Enrichment profiles of the identified pathways are obtained for each molecular subtype.

2.2. Breast cancer biopsy microarray sample set

2.2.1. Primary dataset, cases

A dataset of 819 expression microarrays from untreated, primary breast cancer biopsy samples was compiled and curated from data obtained from GEO. We used microarray data from GSE 4922 (Ivshina et al., 2006), 1456 (Pawitan et al., 2005),7390 (Desmedt et al., 2007), 1561 (Farmer et al., 2005), 2603 (Minn et al., 2005), 2990 (Sotiriou et al., 2006), and 3494 (Miller et al., 2005). All of these sets were analyzed on the Affymetrix HGU133A platform.