STUDY OF THE COMPLEX NETWORKS OF THE LEPTIN SIGNALING PATHWAY IN BREAST CANCER

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Abstract. In this work it is shown that breast cancer is a complex process, due to the large number of molecular factors (genes, receptors, enzymes, proteins, etc.) and environmental factors (tobacco, alcohol, radiation, lack of physical activity, obesity), therefore, an interdisciplinary approach is necessary. Two alternative ways of analyzing and understanding the physical-biological level of the disease are addressed: the experimental part and the theoretical part (theory of complex networks). The first part seeks to understand that the realization of the experiments although they are an important way of explaining how to process occurs (chemical-biological), these generate great costs and can take months or years to simply give us an answer with a reductionist approach. The second seeks to overcome the scheme that places genes and proteins as linear determinants of all phenotypes, to be considered in their global interactions through molecular processes of intracellular-extracellular self-organization, in order to understand systems in less time and with less cost, in addition to using an integrative and systemic approach. This research integrates sufficient evidence to corroborate that the genes are self-organized in these complex networks, whose hierarchical and modular structures allow us to understand the way in which a mutation or removal of specific genes (such as oncogenes and tumor suppressors) can alter global cellular behaviors (proliferation, migration, apoptosis, metastatic processes, etc.). In this sense, this work assumes the challenge of responding to academic, scientific and social needs; This work is intended to be a starting point for new research and to provide information that will help in the treatment and programs to fight against breast cancer.

***Key words**: Breast Cancer, Obesity, Leptin, ObR, Complex Networks.*

INTRODUCTION

Breast cancer is the most frequent neoplasm worldwide with an estimated 1.67 million new cases diagnosed annually; it is the disease with the highest mortality in women with 521,907 deaths per year, which represents 14.9 % of all cancers [1]. In Latin America it is the first neoplasm in women with an incidence of 152,059 cases per year and with a mortality of 43,208 deaths, representing 14% of annual deaths due to this cause. In Mexico in 2014 there were 11,372 new cases of breast cancer, becoming the second cause of cancer death in the country, with an incidence rate of 35.4 per 100,000 women, and an incidence rate of 22.56 % is estimated. of this condition. In 2015, there were 6,250 deaths in women with a rate of 18 deaths per 100,000 women [2].

In developing countries, the incidence of breast cancer is increasing considerably due to changes in reproductive factors, lifestyle and an increase in life expectancy. It is expected that these rising cancer rates in the world will reach a 55 % increase in incidence and a 58 % increase in mortality in less than 20 years [3]. Several epidemiological studies have revealed that obesity and overweight are an important risk factor for the development of different types of cancer such as ovarian, endometrial, prostate, colon and breast cancer. Many of the results obtained from studies suggest that different hormones can act as growth factors and promote the development of breast tissue carcinogenesis; among these hormones is Leptin [4].

Currently, several studies have established an existing correlation between elevated blood levels of leptin and increased body mass index (BMI) [4]. Preclinical studies have shown that both leptin and its functional ObRb receptor are involved in the risk and progression of breast cancer [5]. With the aforementioned, one can visualize the importance of the study of Leptin in breast cancer to identify the mechanism by which Leptin induces the development of malignant cells and thereby identify new therapeutic targets directed against the action of this hormone in the neoplastic cells.

The cancerous and normal cellular phenotype then appears as a physiological property that emerges from networks of interaction between genes, proteins and metabolites that are intrinsically dynamic and that describe the way in which a cell changes in space and time to grow, reproduce, differentiate and all other functions necessary to stay alive. Although the theory of networks applied to the cancerous genotype helps to conceive genes and proteins as a system of self-organized interactions that produce a specific biological function, this only represents a part of the biological complexity of cancer.

We have already seen in the biological foundations of this work, that breast cancer can not be reduced either to linear genetic processes or unicellular models, but must consider the formation of pluricellular systems, as is the tumor itself. The theory of networks has focused on the study of the genome, proteome and metabolome, putting the histological level in the background. However, there are several indications of the emergence of properties such as robustness and

modularity that open up clues for a possible extrapolation of the theory of scale-free networks at the level of histological processes.

METHODOLOGY

1. Obtained data from the Leptin signaling pathway through the platform **STRING®** (https://string-db.org).
2. Three types of networks of the leptin signaling pathway were obtained in the CaMa in STRING®, through the sources of active interaction as follows:

a) The first complex network of Leptin with all active interactions was obtained
(Textmening, Experiments, Databases, Co-Expression, Neighborhood, Gen-Fusion, Co-occurrence).
The active interactions are all the evidences of which STRING® obtained the information of the Leptin signaling path in order to obtain the most complete form of the complex network.

b) The network was obtained with the active interaction type Textmining.
Textmining is based on information retrieval, data collection, statistics and computational linguistics; shows a list of significant protein interaction groups, extracted from the abstracts of the scientific literature.

c) The network was obtained with the type of active interaction Databases.
A database is a collection of schemas, tables, queries, reports, views and other elements; displays a list of significant protein interaction groups, compiled from databases.

3. The complex network was built using the adjacency matrix in the software **GEPHI®** V0.9.1 (https://gephi.org/).

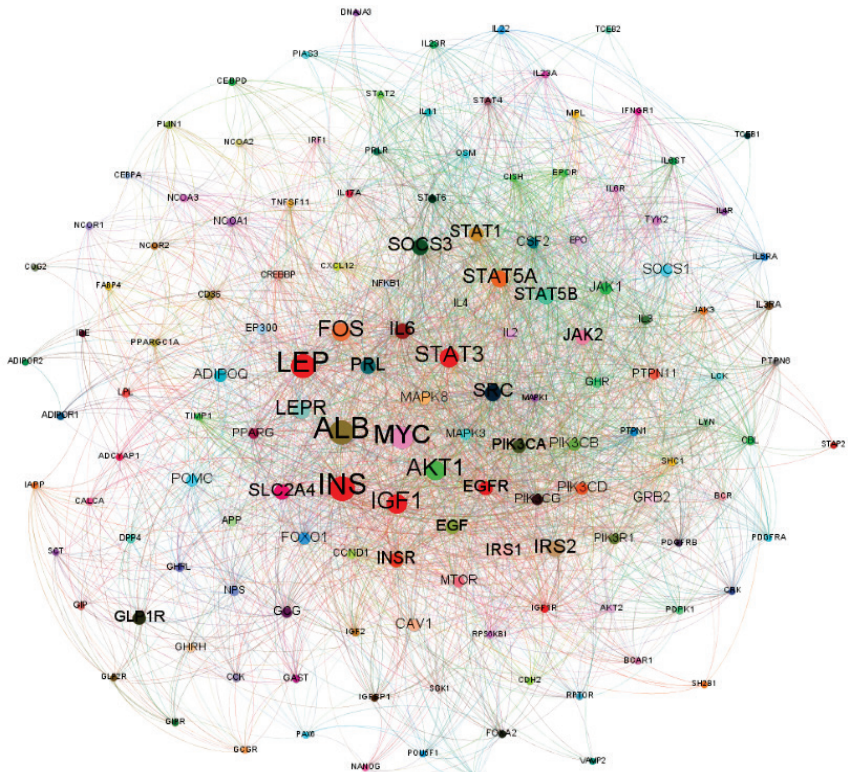
a) After obtaining the list of links and the list of nodes, these were exported in .csv format (comma-separated values) to the GEPHI® network analysis software, to obtain the complex network of Leptin in the CaMa.

4. Once the statistical properties of the network were evaluated, the results were exported in graphml format to the programming language PYTHON® V3.0 (www.python.org). Using the Python® SPYDER interpreter, obtained from the Anaconda distribution, we proceeded to graph the histograms of each centrality property of the complex network of the Leptin signaling pathway.
5. Correlation of theoretical results obtained with the results reported in the literature.

RESULTS

Betweeness Centrality.

The property was evaluated by the procedure described above. Betweeness Centrality of the Directed Network and consigned to the frequency histogram of said property. Once the histogram was obtained, the linear regression was performed to evaluate the power law characteristic of the Betweeness Centrality property (Fig. 1). Looking at table, we can see the 10 main nodes with the highest numerical value of Betweeness Centrality.



Label	Betweeness Centrality
INS	0.01473232
ALB	0.01415062
LEP	0.01401957
MYC	0.01222091
IGF1	0.01039543
AKT1	0.01017011
STAT3	0.00993843
FOS	0.00944907
LEPR	0.00796448
STAT5A	0.00784066

Figure 1. Complex Network of Leptin pathway

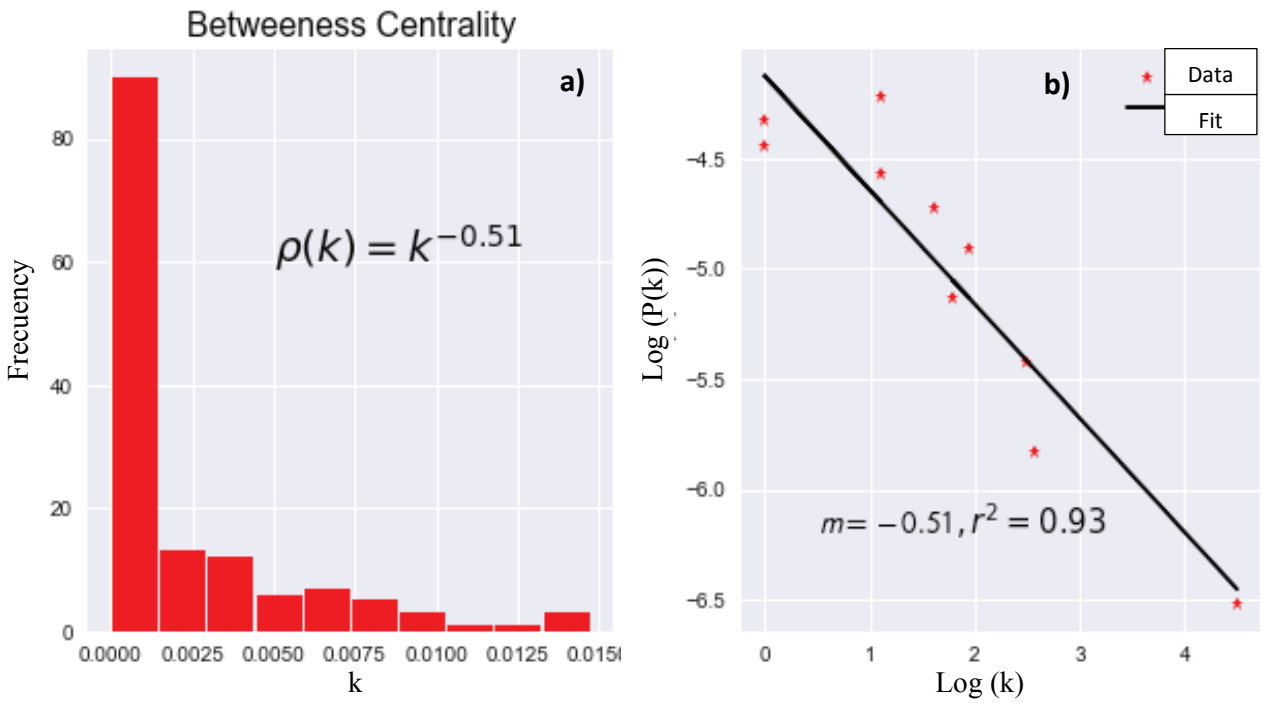


Figure 2. a) Histogram of Betweeness centrality and b) Linear regression for a power law

Figure 2a shows the probability distribution of the Betweeness Centrality property, which presents a power law, an essential characteristic of scale-free networks that are present in most of the metabolic networks and signaling pathways of biological systems. In Figure 2b a linear fit for said property was performed by the least squares method; we can mention that the adjustment is good, since we obtained an r2 = 0.93, which indicates that the adjustment is adequate to measure the linearity.

This structural property is one of the most important from the point of view of complex networks; since the information it provides allows us to know the nodes with more connections in the entire network of the leptin signaling pathway and allows us to predict which nodes are more important in that network and that could represent possible therapeutic targets in breast cancer. The results obtained from these allow us to know the intrinsic properties of the leptin network. In figure 1 it is distinguished that the node with the highest Betweeness Centrality is insulin because the up-regulation of insulin (INS) directly increases the proliferation of cancer cells and breast cancer; in recent studies it has been reported that INS actions can also occur indirectly through a lower availability of globulin and binding proteins (mainly albumin ALB, which is the second node with higher Betweeness Centrality), to similar growth factor insulin (IGF-1) and an increase in the blood concentration of testosterone, estrogen and IGF-1. Elevated concentrations of estradiol and unbound testosterone have been associated with an increased risk of breast cancer in pre and postmenopausal women [1-3]. Insulin also inhibits the production of sex hormone binding globulin (SHBG) and increases the levels of IGF-I in the blood, resulting in increased mitogenic activity [4]. This link is consistent with approximately 50 % of breast cancer tumors that overexpress the IGF-I receptor [5]. Also in figure1, leptin is seen as one of the most important nodes, since the flow of information in that node is of vital importance for the complex network in question. It is known that between leptin and insulin there is a close relationship since they regulate each other. Thus, leptin inhibits the production of insulin in the β cells of the pancreas; while insulin stimulates the production of leptin in the adipocyte, but it can also induce resistance to leptin by inhibiting the signaling of leptin through JAK2 [6]. The signaling pathway activated by ObRb includes the classical cytokine pathway JAK2/STAT3 (Janus kinase2/signal transducer and transcription activator 3). Both leptin and ObRb appear to be significantly overexpressed in cancerous tissue in relation to the noncancerous epithelium. In addition, the identification of the leptin receptor in the β-pancreatic cells suggests the existence of an "adipoinsular" feedback loop through which leptin can inhibit insulin secretion. Therefore, it is proposed that the insulin-leptin axis plays a fundamental role in the pathogenesis and progression of breast cancer.

CONCLUSIONS

When we evaluate the Betweeness Centrality property of the signaling network, it is found that the most important nodes in the network can be identified, this property characterizes the most important nodes based on the shortest paths between pairs of nodes.

1. The laws of power are characteristic of many biological systems, since such phenomena can be identified because they possess scale invariance, that is, fractal properties.
2. The network of the signaling pathway of Leptin has its own power law, which allows us to identify which are the most important nodes in the network.

3. The most important nodes identified so far through the Betweeness Centrality property are: STAT3, LEPR and IGF1 to name a few.

4. The node with the highest Betweeness Centrality is insulin because the up-regulation of insulin (INS) directly increases the proliferation of cancer cells and breast cancer [1].

5. Elevated levels of IGF-I in the blood result in increased mitogenic activity. This link is consistent with approximately 50 % of breast cancer tumors that overexpress the IGF-I receptor [5].

6. IL-4 signaling promotes through immunosuppressive effects on T cells increased proliferation of cancer cells, resistance to apoptosis and major survival of cancer stem cells.

7. This methodology could serve to identify therapeutic targets.

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