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Close link between breast cancer & apoptosis

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Abstract---The mammary gland represents an unique model system to allow investigation of factors that underpin biological initiation of apoptotic processes and related signal transduction pathways. One advantage is that the mammary gland, unlike most other organs, has

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the capacity to go through many cycles of growth, differentiation and anatomical structural development. Indeed, the mammary gland has evolved highly efficient processes (that straddle both lactation and post-lactational phases) which regulate the balance between cell death and proliferation. This paper will discuss the molecular and cellular aspects of apoptosis in the mammary gland with a particular emphasis on the role of apoptosis in breast cancer development.

Keywords--close link, breast cancer, apoptosis.

Mammary gland morphogenesis

The mammary gland undergoes profound rounds of regeneration. Mammary gland ductal morphogenesis is characterized by the formation of branching ducts via rapid epithelial invasion of the stromal fat pad by highly proliferative terminal end bud [1]. Ductal morphogenesis stages in the uterus commence at approximately week 1 in mice, and around 7 weeks in the human fetus and have a primary ductal tree of about 10-15 branches [2-4]. Later, during puberty and controlled by estrogen production, rapidly elongating ductal branches form a ductal network with terminal end buds (TEBs) on the tips of the ducts, and with copious proliferation and formation of fat tissue. The last stage of the ductal morphogenesis occurs during pregnancy; progesterone and prolactin hormone production stimulates a tertiary branching and formation of the lobuloalveolar structures (alveologenesis) that expand throughout the pregnancy, and whilst differentiating during lactation to produce milk. After lactation, the lobuloalveolar structures regress via a cell death process, which aids mammary epithelium to remodel itself to return to its formerstate.

In any tissue exhibiting high levels of cellular proliferation and apoptosis it is essential to have efficient removal of unwanted and damaged cells. In murine models, mammary gland TEB body cells exhibit a very high level of apoptosis, with levels reaching up to 11% during normal ductal development and 4-5% during mammary involution [5].In contrast, the levels of cell death in most quiescent tissues is only 0.1-1%. Also, according to Humphreys et al. the DNA synthesis outside the cell layer of the terminal bud is about 21% yet only 13.8% inside [6].The apoptosis rate reaches up to 14.5% of cells within three cell layers compared to 7.9% outside the lumen. Having said this, cell death and its regulatory mechanisms in ductal morphogenesis remain poorly understood. It has already been amply demonstrated that the increase in cell death is induced by disruption of the interaction between normal epithelial cells and the extracellular matrix (ECM) [7-9]. Degeneration and remodeling of ECM assist ductal branching and reduce cell adhesion [10]. In addition, several proteins are thought to be central to the regulation of apoptosis during ductal morphogenesis [11]. Induction of BH3, a member of the pro-apoptotic Bcl-2 protein family (which also includes Bik, Bad, Bid, Puma and Noxa), has been shown to be involved with induction and regression of apoptosis [12].

Throughout various phases of development, epithelial cell apoptosis represents a regulatory process responsible for control of mammary gland function. Mammary

gland involution is a two-phase process whereby the lactating gland can resume its pre-pregnant state; the secretory alveolar epithelium is reduced from the ductal tree by programmed cell death [14-15]. During the first 2 days of weaning, the initial phase is triggered by stasis of milk. This is reversible and involves an upregulation of pro-apoptotic factors, most particularly caspases, and also reduction in certain survival factors [16]. Under these conditions alveolar epithelium undergoes cell death although the lobular alveolar structure is not remodeled which leaves these tissues relatively unchanged. By these characteristics involution remains reversible, meaning that lactation can restart if suckling is resumed [17]. The predominant pathway acting throughout this is a lysosomal-mediated cell death, whereby enhanced lysosomal membrane permeability leads to the release of cathepsin proteases which, in turn, promote high levels of cell death [18-19].

Apoptosis Pathways

The extrinsic pathway includes several protein members like the death receptors, the membrane-bound Fas ligand, the Fas-associated death domain (FADD), the Fas complexes, and caspases 8 and 10 , which ultimately regulate the downstream caspases rate leading to apoptosis. Activation of the extrinsic pathway is commenced through the ligation of cell surface receptors called death receptors (DRs) , which comprises TNF receptors including TNFR1, DR3 (Apo 2), DR4 (tumor necrosis factor related apoptosis-inducing ligand receptor 1 [TRAIL R1]), DR5 (TRAIL R2), and DR6 [20]. Fas signals play a crucial role in immune surveillance of transformed cells, therefore any defect or mutation in this pathway have been implicated to form many malignancies and autoimmune disease. The Fas ligand (FasL)-Fas system is centered by its death-related functions. Once the stimulation for cell death triggers the pathway, the membrane-bound FasL recruits the inactive Fas complexes and forms the death-inducing signaling pathways. Caspase-8 and -10 induce the death signaling pathways, which first lead to activation of caspase -8 and then, in turn, it activate the rest of the downstream caspases. In some type of cells, the activation of caspase 8 may be the only demand to execute death with some type of cells. Caspase-8 interacts with some intrinsic apoptotic pathways by cleaving Bid (a proapoptotic member of Bcl-2 family) which subsequently release cytochrome-c. Dysregulation of extrinsic pathway leads to malignant transformation whether mutations or deletions of the Fas gene have been seen in some hematologic malignancies [21].

Cancer Development

Cancer is a common, worldwide disease caused by genetic changes in signaling pathways like cell-cycle progression, apoptosis and cell growth. Various signaling pathways such as NF-kb, TGF-β, PI3K/AKT/mTOR, Map Kinase/Erk, Notch, JAK/STAT, Wnt/β catenin could be regulated by mutations in proto-oncogenes affecting cell development and cellular homeostasis. These pathways provide valuable information for accurate identification of cancer biomarkers for future treatments and prevention. During cancer development, genetic and epigenetic alteration directs cells to escape from homeostatic control, proliferate, grow and metastasize to nearby organs [22]. Cancers of lung, skin, breast, liver and pancreas arise in epithelial cells. Mesenchymal tissues are considered the origin

of sarcomas that occur in fibroblasts, myocytes, adipocytes and osteoblasts, whereas, non-epithelial tumors arise in nervous system cells like glioma cells and hematopoietic tissues such as leukemia and lymphoma [23]. Such cellular alterations promote the progression of benign tumors into proliferating cancer in solid tumors [22]. After tumor excursion, the tumor cell misses the access to oxygen and other nutrients hence, leading to the formation of new blood vessels (angiogenesis) that restore the cell's access to oxygen and nutrients [24]. Consequently, tumor cells develop the ability to spread outside their normal boundaries to the nearby organs, entering the circulation to initiate new tumors at other locations (metastasis) thus defining that the tumor is malignant in nature [25]. This illustration of complex cancer associated events proceed in a linear sequence in various ways in individual tumors and between the tumor sites. This sequence also offers a beneficial framework of several signaling pathways that are integrated in the process of cancer initiation and progression [26].(Fig1)

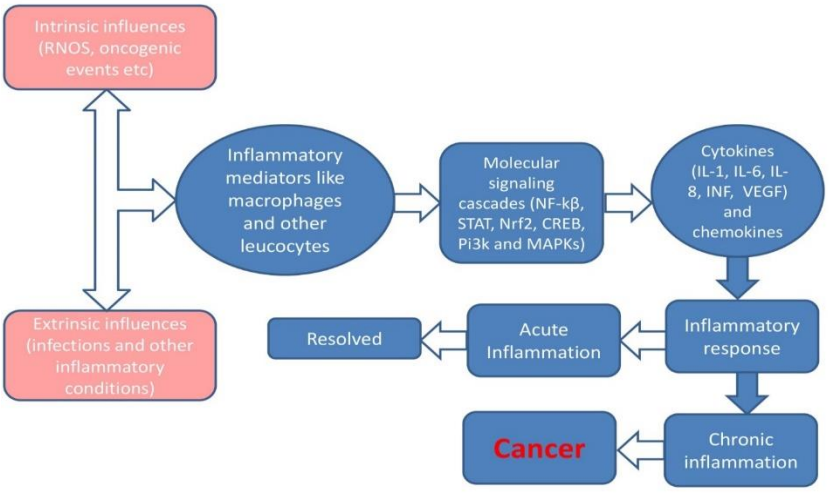

Figure 1. Effect of intrinsic and extrinsic factors on tumor progression

Breast Cancer &Apoptosis defect

The development of normal breast tissue is typically controlled by the balance between apoptosis and cell proliferation. Mohammad et al. described that disrupting this balance will lead to activating the antiapoptotic signal pathway while reducing the proapoptotic pathway, which will eventually trigger uncontrolled cell proliferation in tumor cells [27]. Accordingly, inducing apoptosis represents a crucial approach to mitigate against an excess amount of breast cancer cell proliferation. Several factors increase uncontrolled cell growth in breast cancer cells. These include reactive oxygen species (ROS), DNA damage, growth factor, and UV radiation. Each of these can lead to disrupting the balance between the proapoptotic and antiapoptotic pathways. Rajabi et al. accordingly pointed out that targeting apoptosis can represent an effective method to treat breast cancer [28].

There is mounting evidence which shows that the proliferation of uncontrolled cells in tumor growth is highly affected by reduced apoptosis. The growth or regression of tumors that result in responses to radiotherapy or chemotherapy is determined by the balance between cell proliferation and apoptosis [29]. It has been well established that, in cancer cells, there is inhibition of apoptosis either by the overexpression of antiapoptotic proteins, or by reduced expression of proapoptotic proteins. However, Gandhi, et al. [30] and Lipponen et al. [31] both found that the rate of apoptosis is increased in breast cancer, and this is directly related to poor survival.

In contrast, Keane et al. [32] showed that breast cancer cells were resistant to the apoptosis process. In line with that observation, Herrnring et al. [33] and Singh et al. [34] demonstrated that most breast cancer cells resisted the tumor necrosis factor (TNF) receptor family (which includes CD95 (Fas) and the TNF-related apoptosis-inducing ligands R1 (TRAIL), even with the presence of these ligand receptors in breast cancer cells. Gee et al. [35] showed that the upregulation of antiapoptotic Bcl-2 (B-cell lymphoma 2) family proteins occurred in 80% of breast cancer cases. Whilst this protein works as an antiapoptotic factor, it has been linked with overall high survival rates of breast cancer patients [36-38] whilst, in contrast, the presence of a high rate of apoptotic protein in such patients was linked to a poor prognosis [38]. It seems that an increased level of apoptosis represents the most common defect in breast cancer cells that can lead to worse prognoses and lower survival rates.

We will discuss TWO important genes
First: BRCA-1 and BRCA-2 genes

Breast cancer currently represents a leading worldwide cause of death in women [39], and can best be described as a multifaceted disease. Every cancer carries a genomic mutation of one of the cancer predisposition genes that have been so far identified. BRCA1 and BRCA2 have been determined to be associated with heredity in breast and ovarian cancer [40]. Identifying the mutational profile of breast cancer is important to understand the nature of the diagnosis, and from there, being able to choose the best therapeutic options. The rapeutic intervention at the level of DNA repair pathways has previously been proposed to increase genomic instability and counter BRCA1, BRCA2 mutant, and homologous recombination deficient (HRD) cancer. Breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2) are tumor suppressor genes in which mutant phenotypes predispose to breast and ovarian cancers [41-42]. Both BRCA1 and BRCA2 are large genes that consist of about 100 and70kb, respectively [40].BRCA1 is carried on chromosome 17q21, while BRCA2iscarried on chromosome 13q12. The American College of Medical Genetics and Genomics (ACMG) has issued revised universal guidelines for the interpretation of sequence variants (otherwise known as germline BRCA mutations [40]). Various BRCA1-Interacting proteins have been discovered, all exhibiting different cellular functions. Mutations in the BRCA1 interacting protein DEAH helicase PRIP1are associated with an increased probability of breast cancer [41-42].

Second: p53 & Breast Cancer

Breast cancer can be caused by mutations in P53, which encodes the tumor suppressor protein p53 [43-46]. Overall, invasive primary breast cancers are associated with mutated genes in 30–35% of cases. These mutations are found in around 80% of patients with the triple-negative (TN) form, 10% of patients having luminal A, 30%with luminal B, and the HER2-enriched form represents approximately 70% [47-48].The existence of mutant p53 in breast cancer, particularly the TN subtype, may serve as a biomarker or therapeutic target. In breast cancer, missense mutations dominate, and more than 81% occur in exons 5–8 [49-50]. Exons 4 and 10 have approximately 10% and 6% respectively. It is thought that exons 2, 3, 9, and 11 contain rare mutations (< 2% of cases). Only 19% of mutations are small deletions and 5% are insertions, with most mutations being single-base substitutions (73%) [51]. Mutations in patients with ductal breast cancer are more common than those with the lobular form, and more prevalent in lymph node-positive than lymph node-negative tumors. Furthermore, oestrogen receptor (ER)-negative tumors are more numerous than ER-positive tumors, whereas HER2-positive cases are more prevalent than HER2-negative [49-50].It has been shown that p53 mutations are common in some breast cancers and usually represent an early occurrence [51-54]. The p53 mutation has been observed in 10–30% of ductal carcinomas in situ (DCIS) [55]. There is a good correlation between the point mutation status of matched in situ and metastatic breast cancers [53]. Given this, a p53 mutation can occur before ductal invasion occurs in breast cancer. Additionally, a mutation has been identified in both in situ and surrounding invasive tumor elements, suggesting that the two lesion types are related[56].

Summary

Apoptosis, and the balance between cell death and proliferation, plays a critical role in the initial development of mammary gland tissue. It also represents a vital process that is central to the etiology of breast cancer development. Modulation of apoptosis is controlled by a number of hormones and growth factors, each of which have been shown to influence both breast cancer development, and pathological deterioration. In addition, a number of factors (such as BRCA and p53 mutations), which affect continuous proliferation of breast cancers are closely associated with disease progression.

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