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Molecular Basis of Aging and Breast Cancer

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Abstract

Age is one of the most important risk factors for human malignancies, including breast cancer. In relation to increase in breast cancer incidences, aging can significantly alter breast cancer biology as defined by validated prognostic and predictive biomarkers. Despite awareness that breast cancer and other cancers are primarily age-related diseases, molecular and cellular hypothesis explaining the cancer-aging relationship have only recently emerged and remains clinically unproven. Here we review the series of key observations that has led to complex but growing convergence between our understanding of the biology of aging and the mechanisms underlying breast cancer occurrence.

Keywords: Aging; Breast cancer; Tumour suppressor; Mutation

Abbreviations: ER: Estrogen Receptor; Chk: Checkpoint Kinase; DDR: DNA Damage Response; BRCA: Breast Cancer Associated; FOXO: Foxhead Box O

Introduction

The incidences of malignant tumours increase progressively with age, in both animals and humans. Three major hypotheses have been proposed to explain the association of cancer and age [1-6]. The first hypothesis holds that this association is a consequence of the duration of carcinogenesis which means the high prevalence of cancer in older individuals simply reflects a more prolonged exposure to carcinogens [7]. The second hypothesis proposes the age related progression providing an increasingly favourable environment for the induction of the growth of already existent but latent malignant cells [1,2,8-11]. These mechanisms may also include proliferative senescence, as the senescent cells lose their ability to undergo apoptosis and produce some factors that stimulate epithelial cells with oncogenic mutations [12]. The third hypothesis, which practically joins these two hypotheses, proposes that the cancer prone phenotype of older humans might reflect the combined effects of cumulative mutation load, increased epigenetic gene silencing, telomere dysfunction, and altered stromal milieu [13].

Age is one of the most important risk factors for human malignancies, including breast cancer [14]; in addition, age at diagnosis has been shown to be an independent indicator of breast cancer prognosis. About 80% of all breast cancers occur in women older than age 50. The 10 years probability of developing invasive breast cancer increases from <1.5% at age 40, to about 3% at age 50 and to >4% by age 70, resulting in a cumulative life time risk of 13.2% and a near nine fold higher incidence in women older than age 50 as compared with their younger counter parts [15,16]. ER +ve breast cancers with more general cancer aging postulate that the breast cancer prone phenotype of an older women results from genomic instability and age accumulated mutational loads secondary to telomeric dysfunction and or progressive DNA damage [13]. More consistent with the present evidence is the likelihood that ER +ve breast cancer arising in older women relative to younger women occur by a fundamentally different tumorigenic process manifested more by epigenetic transcriptome differences. More pronounced expression of cell cycle and proliferation associated genes emerged as a strong defining feature of ER +ve breast cancers arising in younger women, perhaps even driving their earlier clinical appearance which an observation consistent with the more aggressive clinical nature of early age-onset breast cancer.

Despite of awareness that breast cancer and other cancers are primarily age-related diseases, molecular and cellular hypothesis explaining the cancer-aging relationship being only recently emerged, remain clinically unproven [17]. In this study, we will provide a molecular framework for understanding the role of cellular aging in breast cancer.

Impact of Aging on Genomic Abnormalities and Transcriptomes in Breast Cancer

It is already shown that in addition to increasing breast cancer incidences, aging can significantly alter breast cancer biology as defined by validated prognostic and predictive biomarkers. In particular, biomarkers thought to reflect breast cancer genomic instability (e.g. high nuclear grade, aneuploidy, p53 immunoreactivity) show strong inverse correlations with patient age-at-diagnosis. Genomic instability is a hallmark of most cancers; it is not too surprising that many of the factors that have been implicated sensing and responding to DNA damage are altered in human tumours. Genomic instability is also a hallmark of aging. A similar age-dependent increase in chromosomal instability has been known to occur in mammals for many years [18]. Recent evidences indicates that the age-dependent accumulation of somatic mutations might vary significantly between different tissues of the same organism and that these genetic alterations might contribute to the stochastic variation in gene expression that is often seen in mammalian aging [19].

On the other hand, six transcriptome subtypes with an apparent age bias (P<0.05) were identified in a hierarchical clustering [20] of 5.1K genes variably expressed in 101 ER-positive RNA samples containing 53 younger women and 48 older women. Samples with poor-outcome associated proliferation signatures were 65% of younger cases. These

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cases involve higher levels of cell cycle associated genes and growth factor amphiregulin (AREG), while older cases involved high level of four different Homeobox Genes (HOX) genes in addition to ER (ESR1). AREG and ESR1 has been proved to be ~80% accurate in differentiating younger cases from older ones confirming age-associated deregulation of ESR1 gene and AREG gene. The molecular analysis of human inherited cancer syndromes such as Li-Fraumeni syndrome, Ataxia-Telangiectasia (AT) and common forms of familial breast and ovarian cancer have strengthened the link between the maintenance of genome integrity and cancer susceptibility. These conditions can be caused by germline mutations in the genes for P53, the Ataxia-Telangiectasia Mutated (ATM) kinase and breast cancer 1 (BRCA1), respectively—three proteins that are essential in the surveillance of DNA damage.

p53

p53 is a quintessential tumour suppressor, the activity of which is lost in nearly half of all human cancer. p53 mutations (exon 5-8) and whole genome aberrations by array comparative genomic hybridization [21,22] show the frequency of wild type p53 vsmut p53 found in ER+ve and ER-ve subsets of early onset and late onset of breast cancer, regardless of tumour stage. The most significant differences to be noted are that late onset breast cancers are 1.5fold more likely to be ER+ve/p53wt and 0.45 fold as likely to be ER-ve/p53wt as compared to early onset breast cancers. While p53 mutations are much less frequently found in ER+ve as compared to ER-ve breast cancers, it is surprising to discover that when ER status is controlled for, p53 mutations are not significantly more frequent in early onset breast cancers relative to late onset breast cancers [23,24].

Human p53 has a Pro/Arg polymorphism at amino acid residue 72 [25]. Humans carrying Pro/Pro genotype had a higher risk of developing cancer compared to the Arg/Arg genotype [26]. In older survivor, p53 Arg protects against cancer more efficiently than p53 Pro but at the cost of a diminished life span [26,27].

BRCA1 and BRCA2 mutation

BRCA1 is a tumour suppressor protein that acts both as a checkpoint protein and DNA damage repair protein [28]. Genomic instability and DNA damage due to BRCA1 deficiency lead to rapid cancer phenotypes in female. Recent findings show that indeed, the ATM or Chk2/p53 DDR pathway is activated upon BRCA1 deficiency in mice. BRCA1 and BRCA2 mutations occur in an early age of breast cancer onset [29].

BRCA1 associated cancers are diagnosed at younger age and are more ER- and PR-, more p53+, and of higher grade than unselected breast tumours or tumours from non BRCA1/2 breast cancer females. However tumours of BRCA2 carrier patients diagnosed at 50yrs or older have more distinctive features and are more ER- and PR-, than tumours of younger patients or tumours of the same age group of BRCA1 patients or non BRCA1/2 patients.

Telomerase dysfunction

Telomeres consist of a tandem repeats of the sequence TTAGGG at the ends of chromosomes and play a key role in the maintenance of chromosomal stability. Telomeres are lost with each cell division cycle due to the incapacity of the replication machinery to copy the terminal sequences of linear templates [30], a problem that is conceivably aggravated by DNA-degrading activities that may operate on telomeres [31,32]. Telomere loss is compensated by telomerase, a reverse transcriptase that adds telomeric repeats *de novo* after each cell division [33]. However adult somatic tissues, including stem cells, do not have sufficient telomerase activity to counteract telomere shortening with aging [30] and consequently, aged organisms accumulate telomere derived chromosomal damage [34,35].

Short telomeres are associated with increased risk of breast cancer [36]. Tumour cells have extremely short and stable telomeres, and their stability is achieved by the activation of telomerase [37,38]. Estrogen may be linked to telomere dynamics through its anti-inflammatory and antioxidant attributes and its ability to stimulate telomerase, a reverse transcriptase that elongates telomere ends [39].

Telomerase is a ribonucleoprotein enzyme that synthesises telomeres in human germ cells, embryogenesis and cancer, maintaining chromosomal length, stability and cellular immortality. Its reactivation appears to constitute a relatively early event in invasive breast carcinogenesis [40-42]. Telomerase activity is associated with high tumour grade, tumour type, nodal metastasis, high cellular proliferation [43], larger tumour size, and lymphovascular invasion in invasive breast cancer [43-47]. However telomerase activity seems to be independent of hormonal receptor status and p53 expression [46] in the above studies. The presence of telomerase RNA template (hTR) and its catalytic component, telomerase reverse transcriptase protein (hTERT) in the serum of breast cancer patients are more evident than in normal breast tissues [48].

Another family of genes that has been intensely studied for its role in aging also seems to have an important function in maintaining genomic stability. This family of proteins is termed the Sirtuins, a name based on the family's founding member, the yeast protein Silent Information Regulator 2.

SIRT

Genes involved in NAD^+- dependent protein deacetylation may have a role to play in cancer pathogenesis [49-51]. These genes comprise of the sirtuins, orthologues of the yeast Silent Information Regulator 2 (SIRT2) families of genes. Sirtuins are a highly conserved set of genes found in organisms ranging from bacteria to man [52,53], involved in a variety of essential cell processes, including aging, preventing differentiation, apoptosis and resistance to metabolic stress [54,55]. Sirtuins, which link the functions of mitochondria, telomere nucleoprotein complex and ribosome production (the MTR), may be important contributors to a wide range of aging related diseases including cancer [56]. Seven Sirtuins (SIRT1-7) have so far been reported in humans. SIRT3 and SIRT7 expressions are increased as primary mammary epithelial cells approach senescence and are also increased in node positive breast cancer in consistence with the MTR hypothesis. Indeed, SIRT7 has recently been shown to activate Pol I encouraging growth and proliferation [57]. The association of elevated SIRT7 expression in node +ve tumours, which have a greater recurrence and poorer survival, suggests that this gene may prove to be a good marker of disease progression and tumour behaviour.

SIRT3 expression; like that of SIRT7, was greater in node +ve breast cancer compared to normal breast tissue. SIRT3 has been shown to be specifically targeted and converted into its active form within the mitochondria [55,57]. Cumulative mitochondrial damage contributes to a fall in relative nicotinamide adenine dinucleotide (NAD) levels and concomitant fall in SIRT3 activity, and is associated with growth arrest, senescence and apoptosis [58]. Analogous to SIRT1 and SIRT2, SIRT3 may also function to provide a growth and survival advantage. Indeed variability of the SIRT3 gene has been linked to survival in the elderly

[59]. Increased expression of SIRT3 seen in lymph node +ve tumours probably contribute to survival of these more aggressive tumours. This indicates the potential utility of sirtuins as prognostic markers in node positive breast cancer.

FOXO

In mammals, ability of FOXO factors to induce cell cycle arrest [60-62], DNA repair [63], and apoptosis [64,65] make them attractive candidates as tumour suppressors [66-68]. Loss of FOXO function may lead to decreased ability to induce cell cycle arrest, leading to tumour development. FOXO3 is deregulated in breast cancer. The presence of cytoplasmic FOXO3 in breast cancer sections correlate with poor survival of breast cancer patients. Similarly, the expression of a constitutively active form of FOXO4 reduces the tumour onset as well as tumour size and progression in nude mice transplanted with cells expressing the HER2 oncogene [68] activation of which triggers the activation of the PI3K-AKT pathway.

FOXO3a plays a critical role in suppressing estrogen dependent breast cancer cell growth and tumourigenesis *in vivo* [69]. FOXO3a inhibits ER-mediated signaling through non-genomic pathway and upregulates the expression of three CDK inhibitors that may result in suppression of tumour growth and tumourigenesis in estrogen-dependent breast cancer cells *in vivo*.

Thus FOXO1, FOXO3, and FOXO4 can prevent tumour progression [66-68]. On the other hand, deregulation of these genes may induce tumourgenesis.

KLOTHO

Klotho has been recently identified as a potent tumour suppressor gene in breast cancer [70]. Klotho is a transmembrane protein that can be shed, acts as a circulating hormone and is a putative tumour suppressor in breast cancer. A functional variant of KLOTHO (KL-VS) contains two aminoacid substitutions F352V and C370S and shows reduced activity. Germline mutations in BRCA1 and BRCA2 substantially increase lifetime risk of breast and ovarian cancers. Yet, penetration of deleterious BRCA1 and BRCA2 mutations is incomplete even among carriers of identical mutations. Among BRCA1 carriers, heterozygosity for the KL-VS allele was associated with increased breast and ovarian cancer risk. Studies in breast cancer cells showed reduced growth inhibitory activity and reduced secretion of Klotho F352V compared with wild type Klotho. These data suggest KL-VS as a breast and ovarian cancer risk modifier among BRCA1 mutation carriers [71]. The presence of KL-VS may serve as a predictor of cancer risk among BRCA1 mutation carriers.

Melatonin

Melatonin is a free radical scavenger which has been reported to be declined over age [72]. According to oxidative stress theory senescent phenotypes occur due to accumulation of oxidative damage to cellular components [73] which make an individual susceptible to develop breast cancer. In fact, breast cancer cells have been reported to develop antioxidant properties for redox regulation *in vivo* [74]. However the curative effect of melatonin on the growth of breast adenocarcinoma has also been reported in female Sprague-Dawley rat [75] suggesting link between aging associated oxidative stress and breast cancer development. Melatonin associated redox regulation can have potential therapeutic importance against development of breast cancer.

P16

The tumour suppressing proteins $p16^{INK4a}$ (product of the INK4a/ARF locus) have been linked to both aging and tumourigenesis in animal models. Derepression of INK4a/ARF, producing $p16^{INK4a}$ overexpression mainly inhibits proliferative property of the cell thereby actually promoting aging due to decrease in regenerative potential of stem cells [76].

ATM

Genetic predisposition, which accounts for ~5-10% of breast cancers, may be a factor during aging. One of the mutagenic events that may result in early-onset of mammary epithelium transformation in form of ionizing radiation is mutation in ataxia-telangiectasia (A-T) gene. The A-T heterozygotes have more cellular radiosensitivity than their wild type counterparts. High dosage of ionizing radiation before puberty has been shown to be associated with development of breast cancer [77]. It has been also reported that A-T gene predisposes for development of breast cancer [78].

SMP30

Senescence Marker Protein 30 (SMP30), also known as Regucalcin, belongs to a novel class of Ca^{++} binding proteins that does not have EF-hand motif. As the name demotes, the protein acts as a significant aging marker, which decreases with age [79]. The protein regulates intracellular calcium homeostasis by enhancing Ca^{++} pumping abilities of plasma membrane. In our recent report [80], we have predicted the association of SMP30 regulation with p53-mediated tumour suppression. Age associated downregulation of SMP30 results in high intracellular calcium as evident from senescent cells. Since SMP30 has been already reported to induce p53 induction [81], this age-mediated downregulation of SMP30 may partially reduce p53-mediated Bax expression under high intracellular Ca^{++} leading to failure of apoptotic induction under any kind of DNA damage.

Differentially Expressed Genes between Early Onset and Late Onset Breast Cancer

To more directly assess the relationship between aging and breast cancer gene copy number abnormalities, comparative genomic hybridization array (aCGH, a 1MB resolution) analyses were performed on tumour DNA samples from two age-based cohorts of breast cancer cases collected from a single geographic region (Bari) and characterized by stage and hormone receptor status (ER, PR). A search for annotated enrichment of the differentially expressed genes for specific biological processes (GO Biological Processes, Expression analysis Systematic Explorer score <0.05) indicated that development and cell cycle/M phase were the most overrepresented functional gene categories. Enrichment of cell cycle associated genes was observed in earlier onset breast cancer while differentially expressed cell cycle/M phase genes represented 20% of all genes overexpressed in the late onset breast cancer. In contrast, the late onset cases showed differentially increased expression of negative cell cycle regulators and four developmentally essential homeobox genes. Two of the overexpressed HOXB genes have been specifically linked to mammary gland development and are known to be expressed in ER+ve breast cancer cells [82,83].

Age-Specific Incidence Rates for All ER/PR Subsets of Breast Cancers

ER-positive/PR-positive, ER-positive/PR-negative breast cancer subtypes increase in rate in postmenopausal years while ER-negative/

PR-negative, ER-negative/PR-positive subtypes decline in incidence rates after age 50 [76]. This data indicates association of ESR1 gene silencing cases with early onset breast cancers which may involve hypermethylation. Interestingly, ESR1 hypermethylation has been shown to be associated with aging of human vascular system [84].

Chromatin Remodeling in Aging May Possibly Lead to Breast Cancer

Aberrant epigenetic gene regulation in aging collaborates with genetic alterations in cancer development [85,86]. Some epigenetic alterations during cancer development include: global DNA hypomethylation linked to genomic instability and the activation of metastasis related genes; DNA hypermethylation in regulatory sequences of DNA repair enzyme genes, tumour suppressor genes, hormone receptors or metastasis inhibitors; alteration in histone acetylation/deacetylation balance that activates genes normally repressed or silences tumoursupressor genes respectively; increased or decreased poly-ADP-ribosylation that affect chromatin structure, cell proliferation, genomic instability, telomere maintenance and ATP dependent chromatin remodelling complexes changes that alter cell cycle control, cell differentiation, and tumour progression. However, modifications in histone methylation, phosporylation, ubiquitinylation and sumoylation also play a role in gene regulation.

The possibility of reversing epigenetic defects provides new targets for therapeutic intervention. Studies on chromatin remodelling in the modification of DNA repair and gene expression are increasing rapidly to design new diagnostic and therapeutic possibilities for cancer. In the respect, it will be of utmost interest to identify those key steps by which it will be possible to reprogram a cancer cell to terminally differentiate or to undergo apoptosis [85-87].

Conclusion

Many striking links exists between advanced age and increased incidence of cancer. Hence we review how several of the age related molecular changes and in particular epithelial carcinogenesis might act in concert to promote cancer. Experimental data indicate that the aged, cancer prone phenotype might represent the combined pathogenic effects of mutation load, epigenetic regulation, telomere dysfunction and altered stromal milieu. Further verification of the role of these effects should in turn lead to the design of effective therapeutics for the treatment and prevention of cancer in the aged.

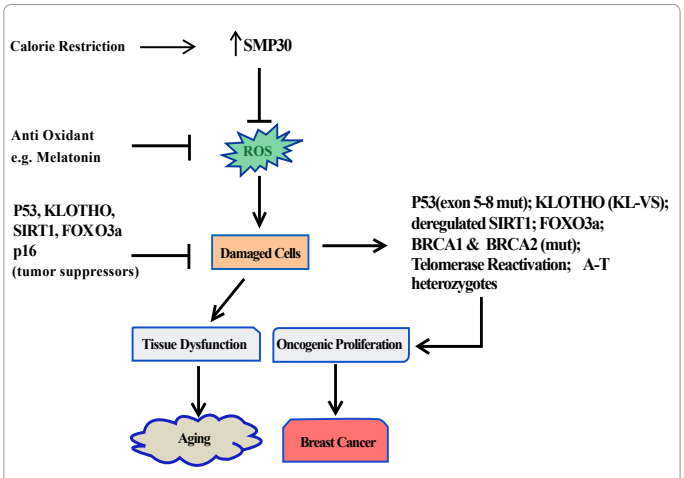

Figure 1: The incidence of cancer increases with aging. A main source of damage for cells originates from the cellular metabolism through the production of reactive oxygen species, ROS, which ultimately cause macromolecular damage, including DNA damage. This endogenous damage is thought to fuel aging as well as cancer. Those mechanisms that diminish to generate endogenous damage are calorie restriction, antioxidant mechanism, p53, SIRT1 and FOXO3a downstream signalling and KLOTHO hormone secretion protect cells from aging and cancer, in the other way their deregulation promote cells to aging and cancer.

The challenge for the future will be to manipulate these mechanisms so that the ongoing enterprise of a longer and healthier life can be continued. In nutshell (Figure 1) FOXO3a may be novel therapeutics for inhibition of tumour proliferation and development in breast cancer. Specifically, molecular changes in SIRT3 and SIRT7 expression may contribute to tumour development and disease progression. Study of sirtuins has a potential application in breast cancer diagnosis and prognosis, as well as in understanding disease biology. Telomerase RNA may serve as a useful cancer marker in future. Klotho status may serve as an identifier of carriers who are at increased risk of cancer development. BRCA1 and BRCA2 mutations are found in early age of breast cancer on set. p53 mutations are much less frequently found in ER+ve as compared to ER-ve breast cancer, p53 mutations are significantly found in late onset breast cancer as compared to early. Melatonin may be useful as a potential free radical scavenger with progressive aging in order to inhibit development of breast cancers.p16 may prove to be an effective tumour suppressor.ATM may serve as a marker gene for predicting susceptibility of the breast tumour towards ionizing radiation. Klotho is an age associated gene, its functional variant status acts as an identifier of carriers who are at increased risk of breast cancer. Likewise, status of SMP30 in breast cancer may also predict involvement of p53 and calcium homeostasis and thus have prognostic value. Further research is needed to identify other age associated genes which will help us study the prognosis of breast cancer.

One of the risk factors for developing cancer is age. The exponential rise in cancer incidence with age has been explained by the accumulation of a critical number of mutations, primarily in the epithelial cells that give rise to the majority of malignant tumours in adults. Therefore we should encourage aging research, to identify new genes that are differentially expressed in cancer as well as in aging and broadly study their molecular mechanisms to develop new therapeutic approaches to cancer.

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