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Critical Reviews in Oncology/Hematology xxx (2007) xxx–xxx

CRITICAL REVIEWS IN Oncology Hematology Incorporating Geriatric Oncology

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Impact of aging on the biology of breast cancer

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Accepted 5 September 2007

Contents

Abstract

Breast cancer is a heterogeneous malignancy; its age-specific incidence profile rises exponentially until menopause and increases more slowly thereafter, reflecting the superimposition of early-onset and late-onset breast cancer rates. While early-onset breast cancers largely represent inherited or early life transforming effects on immature mammary epithelium, late-onset breast cancers likely follow extended exposures to promoting stimuli of susceptible epithelium that has failed to age normally. Among stimuli thought to promote late-onset breast tumorigenesis are the altered extracellular matrix and secreted products of senescent fibroblasts; however, the extent to which these senescent influences exist within the aging breast remains unknown. Clinical observations and biomarker studies indicate that late-onset breast cancers grow more slowly and are biologically less aggressive than early-onset breast cancers, even when controlled for hormone receptor (e.g. estrogen receptor, ER) and growth factor receptor (e.g. HER2) expression, supporting the conclusion that the biology of breast cancer is age-dependent.

Keywords: Age-associated malignancies; Breast cancer; Mammary gland involution; Cellular senescence; Prognostic and predictive biomarkers; Estrogen receptor

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doi:10.1016/j.critrevonc.2007.09.001

Please cite this article in press as: Benz CC, Impact of aging on the biology of breast cancer, Crit. Rev. Oncol./Hematol. (2007), doi:10.1016/j.critrevonc.2007.09.001

1. Breast cancer: a heterogeneous age-associated malignancy

The vast majority of human malignancies are age-associated cancers, showing incidence rates that increase exponentially with age during adulthood such that over 75% of all invasive cancers occur in susceptible populations age 55 years or older [1]. Cancer incidence in the United States (US) has been monitored since 1973 by the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI), which now collects data from 18 different SEER registries representing ~26% of the US population and reports incidence and survival rates per 100,000 adjusted to the US population's age distribution in the year 2000. While overall age-adjusted US cancer incidence rates have increased about 15% over the past three decades, breast cancer age-adjusted incidence rates have increased nearly 23% to a current level of ~130 cases per 100,000, representing ~180,000 new cases yearly [2]. Unlike the age-specific incidence profile for all cancers, that for invasive breast cancer or its precursor lesion, ductal carcinoma in situ (DCIS), shows an exponential rise until menopause (about age 50) followed by a slower rate of increase, as shown by the 1992–1997 SEER incidence curves in Fig. 1 (panel A). Consequently, about 80% of all breast cancers arise in women over age 50; and the 10-year probability of developing invasive breast cancer increases from less than 1.5% at age 40, to about 3% at age 50 and over 4% by age 70, producing a cumulative lifetime risk of 13.2% or 1 in 8 [3]. When the SEER incidence data shown in panel A of Fig. 1 are broken into four clinical breast cancer subsets based on registry reported estrogen and progesterone receptor (ER, PR) status, four different age-specific breast cancer incidence curves are revealed, as shown in panel B of Fig. 1 [4]. Notable are the near identical increases in age-specific incidence rates for each of the four ER/PR subsets during premenopausal years and the markedly different curve inflections near age 50; only the two ER-positive breast cancer subtypes (ER-positive/PR-positive, ER-positive/PR-negative) show ever increasing rates during postmenopausal years, while ER-negative breast cancers (ER-negative/PR-negative, ER-negative/PR-positive) show a slight decline in incidence rates after age 50. The classically recognized inflection point about menopause ("Clemmesen's Hook") in the overall age-specific breast cancer incidence curve is now known to reflect the superimposition of two different rate curves, an early-onset type breast cancer with a modal age of diagnosis at ~50 years and a late-onset type breast cancer type with a modal age of diagnosis at ~70 years. Over 270,000 SEER registry breast cancer cases diagnosed across the US between 1992 and 2002, with known stage and steroid receptor status (ER, PR) and charted for seven different histopathologic invasive subtypes (ductal, tubular, lobular, medullary, inflammatory, papillary, mucinous) and three different racial origins (White, Black, Asian or Pacific Islander), were analyzed by age–density plots and a statistical mixture model to reveal that a bimodal age distribution provides a better overall fit to the incidence data than a single age density distribution model [5]. High-risk tumors (large size, positive lymph nodes, high grade, negative ER

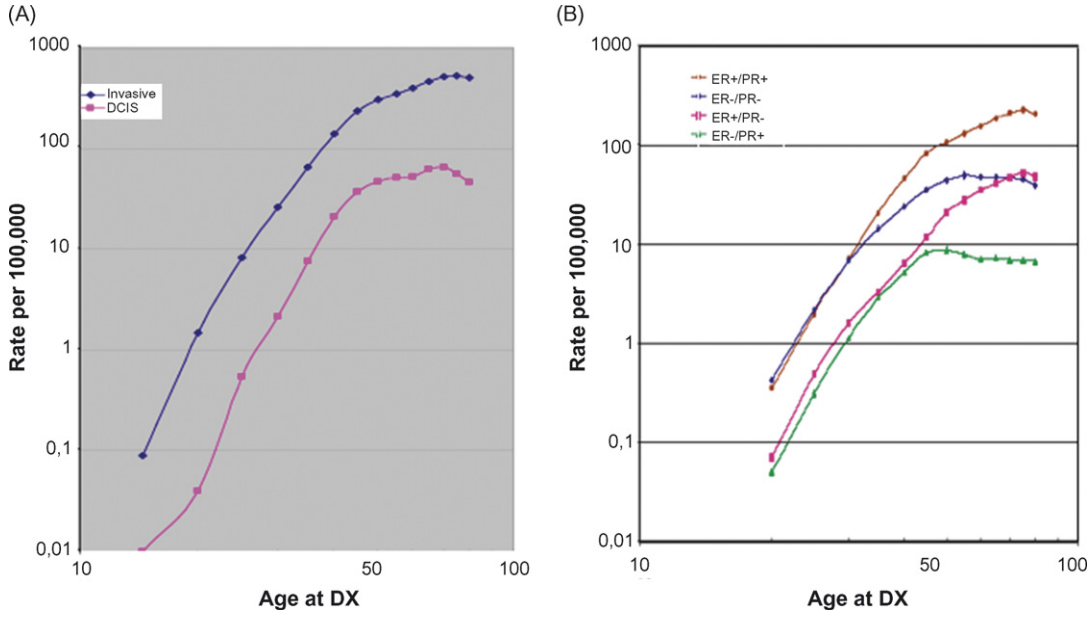

Fig. 1. Age-specific incidence curves (log–log plots) for overall invasive and ductal carcinoma in situ (DCIS) newly diagnosed breast cancers (panel A), and for invasive breast cancer subsets according to ER and PR status (panel B). SEER incident rates (per 100,000, including all ethnic groups) for 5-year age groups determined from the SEER reporting interval 1992–1997. Known ER and PR status was available for over 80,000 cases across all age groups; these cases consisted of 62% ER-positive/PR-positive, 13% ER-positive/PR-negative, 4% ER-negative/PR-negative, and 21% ER-negative/PR-negative. Data and figure revised from previous publication [4].

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and PR) show predominantly early-onset age distributions while low-risk tumors (small size, negative lymph nodes, low grade, positive ER or PR) show predominantly late-onset age distributions at diagnosis [5]. With the exception of medullary breast cancer, which fits an early age unimodal model across all racial groups regardless of steroid receptor status, all histopathologic breast cancer subtypes demonstrate similar bimodal age density distributions within each racial group. As well, when this statistical method was applied to a separate published set of breast cancer cases molecularly subclassified by gene expression microarrays (total 122 Stanford/Norway cases) into luminal (subtypes A and B) or non-luminal (basal and HER2-positive) types, both molecular types exhibited bimodal age-at-diagnosis distributions, with the luminal cases appearing most like ER-positive late-onset breast cancers and the non-luminal cases appearing most like ER-negative early-onset breast cancers [5].

2. Normal mammary gland changes with aging and menopause

While aging is highly individualized, normal age-related changes occur in organs that are also at risk for malignant transformation. Whether these normal age-related changes represent a shifting tissue background from which malignancy must be differentiated or in some way contribute to the tumorigenic process is a fundamental question under intense investigation. Normal organ-specific aging may entail diminished tissue mass and function (e.g. liver, kidney, skeletal muscle), loss of functional reserve without substantial loss of tissue mass (e.g. cardiac muscle, lung, gastrointestinal tract, brain, marrow and immune cells, most exocrine and endocrine glands), or tissue remodeling with altered organ function (e.g. male and female reproductive glands). In non-pregnant women, ovarian size and function diminish progressively after the second decade of life, uterine size peaks by the fourth decade and then declines, and breast glandular mass is progressively lost and replaced by a combination of fatty tissue and collagenous stroma [6,7]. The molecular and cellular effects of aging on normal breast tissue are, therefore, superimposed on a continuum of developmental changes in mammary gland epithelium that normally occur between puberty and menopause, heavily influenced by menstrual history and parity. For women it is often difficult to distinguish the effects of normal aging from those of natural menopause, or the earlier incipient decline in ovarian estrogen (E) production with its resultant effects on ER expressing target organs like the breast. Expression of ER in the normal breast shows a gradual >3-fold increase beginning in the third decade and plateauing by the sixth decade of life [4]. In contrast, estrogen-inducible proteins like PR show no significant age-specific change in their average level of expression in the normal breast, although they are certainly subject to monthly changes within each menstrual cycle. Further complicating age-related influences on the normal mammary gland is the marked but variable age-related increase in breast adipose and stromal cell production of the enzyme, aromatase, encoded by the gene *CYP19A1*. Androstenedione and testosterone, whose serum levels in postmenopausal women are not much reduced from those in follicular phase premenopausal women, are the androgenic precursors converted by aromatase into estrone (E1) and estradiol (E2), respectively. While postmenopausal serum estrogen (E1 and E2) levels are markedly reduced relative to premenopausal serum levels, the age-related increase in mammary gland aromatase production is such that postmenopausal mammary gland estrogen levels can approach those of a premenopausal mammary gland [8].

3. Tumorigenic predisposition within the aging mammary gland

Despite longstanding awareness that breast and other cancers are primarily age-related diseases and that aging predisposes to diseases like cancer, geroscience is still in its infancy [9] and is only beginning to inform oncology about the cancer–aging relationship [10]. Consequently, emergent molecular and cellular hypotheses put forth to explain the cancer–aging relationship are of interest but remain largely untested [11].

3.1. Timing of carcinogenic events

One obvious aspect of this relationship involves the time and number of premalignant steps required between mutagenic initiation and complete tumor promotion to generate a clinically apparent cancer. Studies of human breast cancer latency after a mutagenic dose of ionizing radiation or inheritance of a breast cancer predisposition gene (e.g. mutated *BRCA1*, *BRCA2*, *TP53*, *ATM*, or *PTEN*) indicate that clinical presentation generally requires decades of tumor promotion and growth. Early-onset type breast cancers showing a modal age of diagnosis at ~50 years, as determined from age-specific incidence curves, are thought to largely represent inherited or early life transforming events affecting the immature mammary epithelium [5]. In contrast, later age-onset cancers can emerge in any organ with a replicating cell subpopulation hit by an early mutagenic initiating event and then subjected to prolonged later life exposure to an exogenous or endogenous promoting agent. This later life tumor promotion can also become manifest by age-associated impairments in xenobiotic detoxification, macromolecular repair, immune surveillance or wound healing. With specific regard to breast cancer, both exogenous administration of hormones at menopause (e.g. estrogen and progesterone replacement during menopause) and specific polymorphisms in endogenous steroid hormone metabolic pathways are associated with later age predispositions to breast cancer [12,13].

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3.2. Persistence with aging of breast epithelium susceptible to transformation

Another key aspect of the cancer–aging relationship involves the impact of development and aging on the persistence of replicating (or replication-competent) cell populations that are most susceptible to malignant transformation. For breast tumorigenesis, initiating events must occur relatively early in life since it is known that breast irradiation after age 35 fails to increase subsequent breast cancer risk, implying later-life loss of breast epithelium susceptible to full malignant transformation [12]. Epithelial cells within the human and rodent mammary gland known to be most susceptible to oncogenic transformation are the replicating and hormonally responsive subpopulations within undifferentiated terminal duct lobular units, which are normally reduced in number with increasing age and parity [14]. In a tissue reorganizing process distinct from postlactional mammary gland involution and not coincident with menopause, aging in the normal mammary gland is associated with a progressive reduction in the number and size of breast lobule acini. This loss of acinar epithelium is referred to as age-related lobular involution and is also associated with gradual replacement of the delicate intralobular stroma by a more dense collagenous breast stroma combined with variable amounts of breast fatty tissue [7]. Since the extent of age-related lobular involution is associated with a markedly reduced age-specific breast cancer risk, it has been suggested that breast cancer predisposition is closely linked to the failure of breast tissue to age and involute normally [15]. Unfortunately, the subcellular and molecular mechanisms regulating age-related mammary gland involution are still unknown; presumeably this tissue remodeling process involves some combination of programmed epithelial apoptosis and/or senescence, mechanisms for which there is a growing body of knowledge and increasing evidence of linkage to both aging and cancer [16].

4. Cellular mechanisms linking aging and cancer

Geroscientists contend that among the causes of aging in mammals (and virtually all multicellular organisms) are evolutionarily conserved cellular responses designed to protect an organism from developing cancer. These genome-protecting and tumor suppressing mechanisms include apoptosis and senescence, cellular responses that effectively eliminate or prevent proliferation of genomically damaged but otherwise replication-competent somatic cells at risk for neoplastic transformation [16]. The tumor suppressing proteins p53 and $p16^{INK4a}$ (product of the *INK4a/ARF* locus) have been linked to both aging and tumorigenesis in animal models [17,18]; and it has recently been argued that these two key regulators direct convergent and divergent cellular mechanisms, respectively, that evolved to protect against cancer and aging [19]. Convergent mechanisms, including improved metabolic efficiency, antioxidant defenses and p53 transcriptional responses, act to diminish cellular damage and simultaneously protect against cancer and aging; divergent mechanisms, including telomere shortening and derepression of *INK4a/ARF* (producing $p16^{INK4a}$ overexpression), act mainly to reduce cellular proliferation and prevent tumorigenesis but in so doing promote aging by limiting the regenerative potential of stem cell populations [19]. Studies of human aging syndromes (progerias) suggest that various other genes also regulate both aging and cancer [20]. None of the well-studied progeroid syndromes (Hutchinson-Gilford, Werner, Bloom, Rothmund-Thomson, Cockayne, dyskeratosis congenita, trichothiodystrophy) are thought to perfectly represent precocious total body aging; in fact, many involve only "segments" of body aging and are thus referred to as segmental progerias. Genotoxic stress in the form of unrepaired DNA damage, caused by physical (e.g. UV, X-rays) or chemical (e.g. reactive oxygen species) agents, some from our own cellular metabolism, can produce DNA mutations and chromosome aberrations that lead to cancer and/or trigger cell senescence or apoptotic mechanisms that promote aging by causing functional decline and loss of organ or tissue cellularity. The rate and type of DNA damage (e.g. single strand or double strand breaks, base adducts or interstrand crosslinks) as well as a cell's ability to respond and repair this damage determine the cellular and organismal consequences: cancer, aging or both. An intricate network of repair systems have evolved to address specific subclasses of DNA damage (e.g. nucleotide and base excision repair, transcription-coupled repair, homologous recombination and non-homologous end-joining to fix double strand breaks), producing a fine balance between anti-cancer and anti-aging protection mechanisms [21]. When unrepaired in proliferating cells, DNA damage may be either diluted out by replication or propagated into mutations and chromosome aberrations within daughter cells, resulting in malignant transformation; when unrepaired in non-dividing (postmitotic) cells, DNA damage may gradually accumulate until cell death or senescence ensues. Varying disturbances in the balance between anti-aging and anti-cancer genome maintenance mechanisms are apparent in the different progeroid syndromes. Those syndromes associated with increased risk of malignancy (e.g. Werner, Bloom, Rothmund-Thomson, xeroderma pigmentosa, dyskeratosis congenita) often result from inherited mutations in genes involved in global repair systems (e.g. DNA helicases), generating increased genomic mutagenesis. In xeroderma pigmentosa, for example, the resulting increase in mutagenesis produces a 1000-fold propensity for skin cancer formation yet only minor symptoms of premature aging. In contrast, syndromes with genetic defects in more localized DNA repair systems that do not prevent mutations but promote cell death or senescence responses (e.g. Cockayne syndrome and trichothiodystrophy) exhibit many symptoms of premature aging but may be associated with a decreased likelihood of cancer [21]. Curiously, the ratio between cancers of epithelial origin and sarcomas of mesenchymal organ in the general population is about 10:1, yet in conditions

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like Werner syndrome this ratio is 1:1 suggesting that mesenchymal tissues are more susceptible than epithelial tissues to the consequences of an inherited deficiency in genome maintenance. It is also puzzling that the genes mutated in Werner (WRN) and other progeroid syndromes have not been observed to be mutated or lost in either inherited or sporadic forms of breast cancer [20].

4.1. Do late-onset breast cancers derive from senescent stroma or epithelium?

Cellular senescence was first described over 40 years ago as a process limiting the proliferation of normal human cells. Today, this specific phenomenon is termed replicative senescence and is thought to be triggered by progressive telomere shortening. However, other stressful stimuli (e.g. DNA damage from ionizing radiation or drugs, inappropriate mitogenic signaling, oxidative stress) can also readily induce the senescent cell phenotype, triggered by $p16^{INK4a}$ and/or p53 activation. The senescent cell phenotype is generally described as irreversible proliferation arrest with resistance to apoptosis and altered cell function, including increased secretion of degradative enzymes, inflammatory cytokines and growth factors [16]. It has been proposed that senescent cells slowly accumulate with age; indeed, cell senescence is thought to contribute to aging while protecting from tumorigenesis [16,22]. Thus, to fully transform a population of senescent epithelial cells, the tumor suppressing functions of $p16^{INK4a}$ and p53 must be bypassed. Certainly telomere attrition is observed as an early event in breast tumorigenesis, correlating with increased genomic instability; however, there is considerable variation in telomere length among fully formed breast cancers, and the silencing of $p16^{INK4a}$ and/or mutation of p53 are seen in only a proportion of all breast cancer cases [23]. Primate studies have shown that senescent fibroblasts accumulate with advancing age, however, in postmitotic tissues there is little evidence of age-related senescence [24]. The extent to which senescent fibroblasts or epithelial cells accumulate within an aging breast is presently unknown; but there is ample experimental evidence to suggest that an aging stoma can promote breast tumorigenesis, largely by remodeling the extracellular matrix and promoting invasion and growth of premalignant epithelial cells exposed to the secretory products of the senescent fibroblasts [16,25,26]. Thus, it seems ironic that while the senescence response appears designed to protect a cell population from malignant transformation, senescent stroma can promote tumorigenesis of neighboring premalignant or malignant epithelium. Further insights are needed into the multi-faceted cancer–senescence relationship, as more recent experimental evidence suggests that tumors formed in the absence of functioning p53 can be ablated by reactivation of p53, which induces tumor cell senescence sufficient to arrest tumor growth followed by macrophage and immune cell induced tumor cell destruction [27].

4.2. Do cancer–aging hypotheses predict clinical breast cancer behavior?

Observations of age-dependent deterioration in genome integrity along with increased gene silencing by promoter methylation continue to fuel speculation that genetic and epigenetic aging events drive the increasing cancer incidence of later life [11]. Normal human aging appears to be associated with telomere shortening and increased genomic instability, global and promoter-specific epigenetic changes, and altered expression of genes involved in cell division and extracellular matrix remodeling [28–31]—characteristics shared by many epithelial malignancies like breast cancer. Thus, cancers increased with aging are thought to possess a "mutator" phenotype predisposing to genetic instability, accelerated proliferation, and a generally more invasive and metastatic phenotype [11]. From a clinical perspective, however, there is little direct evidence to support this mechanistic paradigm; and for breast cancers in particular, there is definitive evidence to the contrary. Clinical observations in older patients indicate that their tumors grow more slowly and are biologically less aggressive [32,33]. Also, early-onset breast cancer is known to be clinically more aggressive than late-onset breast cancer [5]; and younger age (<45 years) has been shown to be an independent risk factor for early breast cancer recurrence and death [34,35]. To confirm such observations within a histologically identical group of early-stage ER-positive breast cancers, we turned to a colleague (A. Thor, MD) possessing a well studied archive of >800 breast cancers associated with 18+ years of clinical follow-up and fully characterized by various prognostic markers [36]. Selecting for untreated ER-positive node-negative ($T_{1/2}$) ductal carcinomas diagnosed before age 46 or after age 69 yielded only 83 eligible cases (21 early-onset, 62 later-onset). However, as shown by the Kaplan–Meier plots in Fig. 2, long-term disease free survival (DFS) was significantly different between the two age groups, with 10-year DFS plateauing at <30% of the early-onset group and >70% of the later-onset group ($p = 0.0004$). Adjusting for differences in tumor grade and proliferative index (Ki67/MIB-1) between the two ER-positive tumor groups failed to eliminate the significant outcome differences, supporting the contention that unknown biological features determine the different clinical behaviors of histologically similar early-onset and late-onset breast cancers.

5. Biological differences between early-onset and late-onset breast cancers

To test the premise that breast cancer biology is age-dependent, we performed a retrospective analysis on nearly 4000 primary breast cancers, derived from two geographically different archives (American/MGH, Swiss) and previously characterized with respect to multiple validated prognostic and predictive biomarkers [36]. The paraffin

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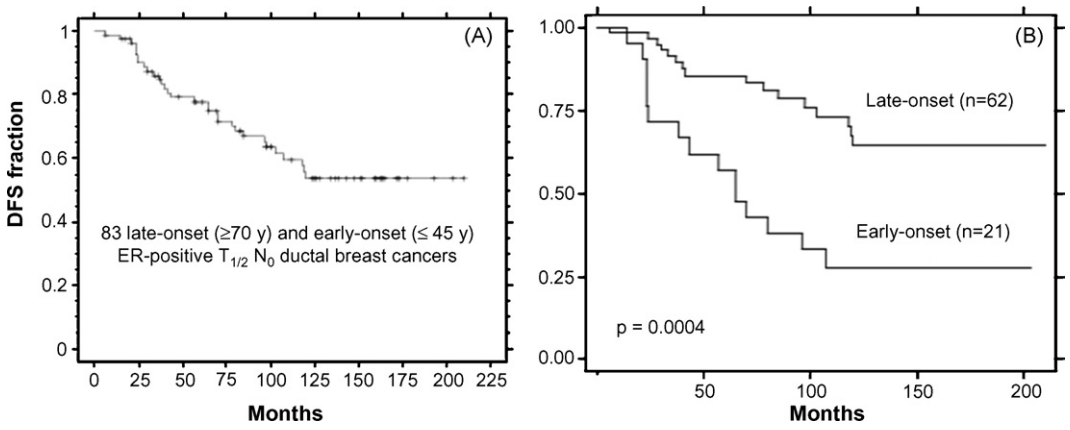

Fig. 2. Kaplan–Meier disease-free survival (DFS) curve for combined set of 83 ER-positive node-negative ductal breast cancer cases untreated with adjuvant therapy (panel A), and DFS curves for late-onset ($n = 62$) and early-onset ($n = 21$) subsets (panel B). As described in the text, the selected age cohorts were well matched for numerous tumor characteristics and biomarkers and differed only by mean tumor proliferation index and high tumor grade. The significant difference in DFS outcomes shown ($p = 0.0004$) could not be eliminated by adjusting for subset differences in tumor grade and proliferation index. Primary data provided by A. Thor and analyzed by D. Moore.

archived American samples were analyzed for histology, tumor grade, stage (TNM), apoptotic and mitotic indices, and were immunohistochemically scored for Ki67/MIB-1, p53, ERBB2, EGFR, ER, PR, and pS2. In addition to scoring tumors by histology, grade and stage, protein extracts from the cryobanked Swiss samples were quantitatively analyzed by immunoassays for ERBB2, EGFR, ER, PR, pS2, Bcl2, VEGF, uPA, uPAR, PAI-1, and cathepsin D. In aggregate, these biomarkers represent surrogate measures of tumor (i) growth, proliferation, and genetic instability, (ii) angiogenic, invasive and proteolytic potential, and (iii) endocrine dependence. Findings from both archives demonstrated that late-onset breast cancers have slower growth rates, are genomically more stable and more likely to be ER-positive, and are less likely to be ERBB2-positive or EGFR-positive. Altogether, they support the conclusion that the biology of breast cancer is age-dependent; however, they do not account for the strong inverse interactions observed between ER and the other age-dependent biomarkers.

5.1. Inverse age relationship between ER and measures of breast cancer growth and genome stability

All surrogate measures of tumor growth and genetic instability showed strong inverse correlations with ER and patient age at diagnosis, when evaluated on a decade-by-decade basis [36]. As shown in Fig. 3, across both archives and whether evaluated quantitatively (panel A) or immunohistochemically (panel B), overexpression of the ERBB2 growth factor receptor declined significantly after age 40, while total ER content and the proportion of ER-positive breast cancers increased continuously after age 40. A similar relationship was seen for the EGFR growth factor receptor. Relative to aging normal mammary gland tissue, these age-dependent changes in breast cancer ER content (fmol/mg protein) mirrored 10-fold lower increases in normal mammary gland ER content up to age 60, rising faster thereafter and reaching a near 25-fold differential between malignant and normal breast tissue by age 80 [4,36]. Also showing inverse relationships to ER content, breast cancer p53-positivity and apoptotic index declined fastest after age 50, while grade, mitotic index and Ki-67/MIB-1 declined most rapidly prior to age 60 [36]. These age-dependent biomarker changes seen in nearly 4000 unselected breast cancer cases were therefore consistent with both clinical and epidemiological evidence indicating that early-onset breast cancers are more aggressive than late-onset breast cancer cases [5,34,35]. Furthermore, they clearly demonstrated the strong inverse age relationships between breast cancer ER content and all surrogate measures of breast cancer growth and genetic instability.

5.2. Aging and measures of breast cancer invasiveness and angiogenesis

Analysis of both breast cancer archives indicated that after age 40 there was no consistent age relationship with tumor stage (TNM staging), nodal involvement, or risk of distant metastasis (M1 stage) at the time of diagnosis [36]. Validated prognostic and predictive biomarkers associated with subsequent risk for local, regional or systemic dissemination include the angiogenic growth factor, VEGF, and the secreted proteases, uPA and cathepsin D. As illustrated in Fig. 4 (panel A), none of these surrogate measures of invasive or metastatic potential showed any significant change when analyzed on a decade-by-decade basis in breast cancer cases diagnosed after age 40, although tumor VEGF levels were on average two-fold higher in tumors arising before age 40 than in those arising after age 40 [36]. While expression levels of these biomarkers mediating breast cancer invasiveness and angiogenic potential did not change significantly with increasing age, a more recent study suggested that similar tumor expression of VEGF or uPA might be associated with significantly

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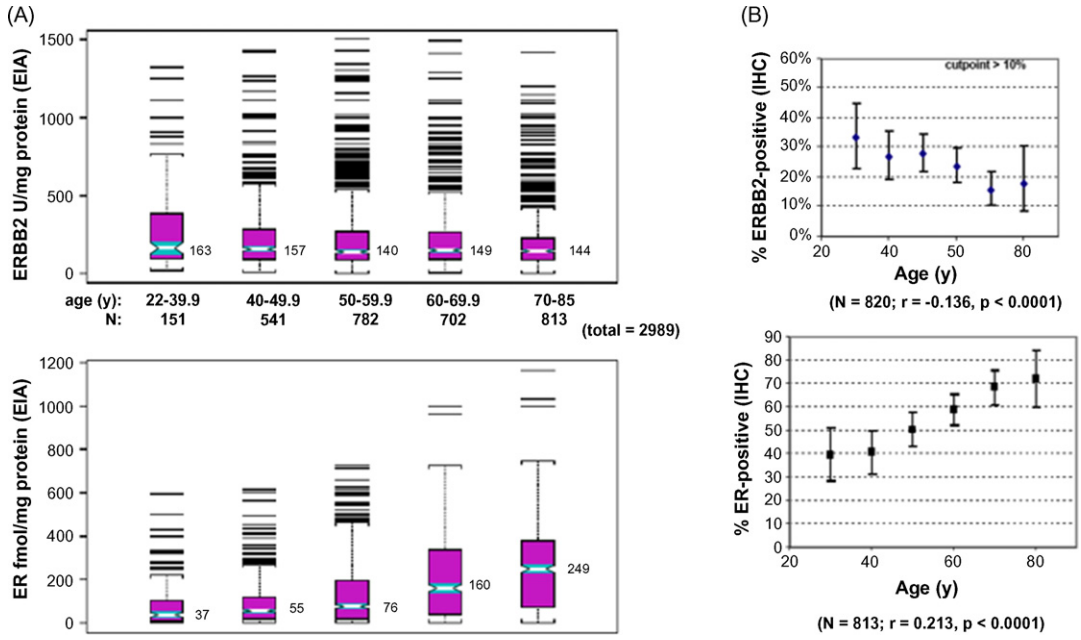

Fig. 3. Age associations for ERBB2 and ER content (panel A) or their percent overexpression (panel B) for unselected primary breast cancers from two different archives. Cryobanked Swiss tumor extracts ($n = 2989$) were analyzed by quantitative enzyme immunoassays (EIA), while formalin-fixed paraffin-embedded American/MGH samples ($n > 800$) were analyzed by immunohistochemistry and scored for percent positive staining tumor cells. Notch-boxplots show median values for each age group. Proportion plots show median% values (±95% confidence intervals) for each age group, with linear regression fit (r, Pearson's correlation coefficient) and statistical significance (p values) indicated below. Figures modified from previous publication [36].

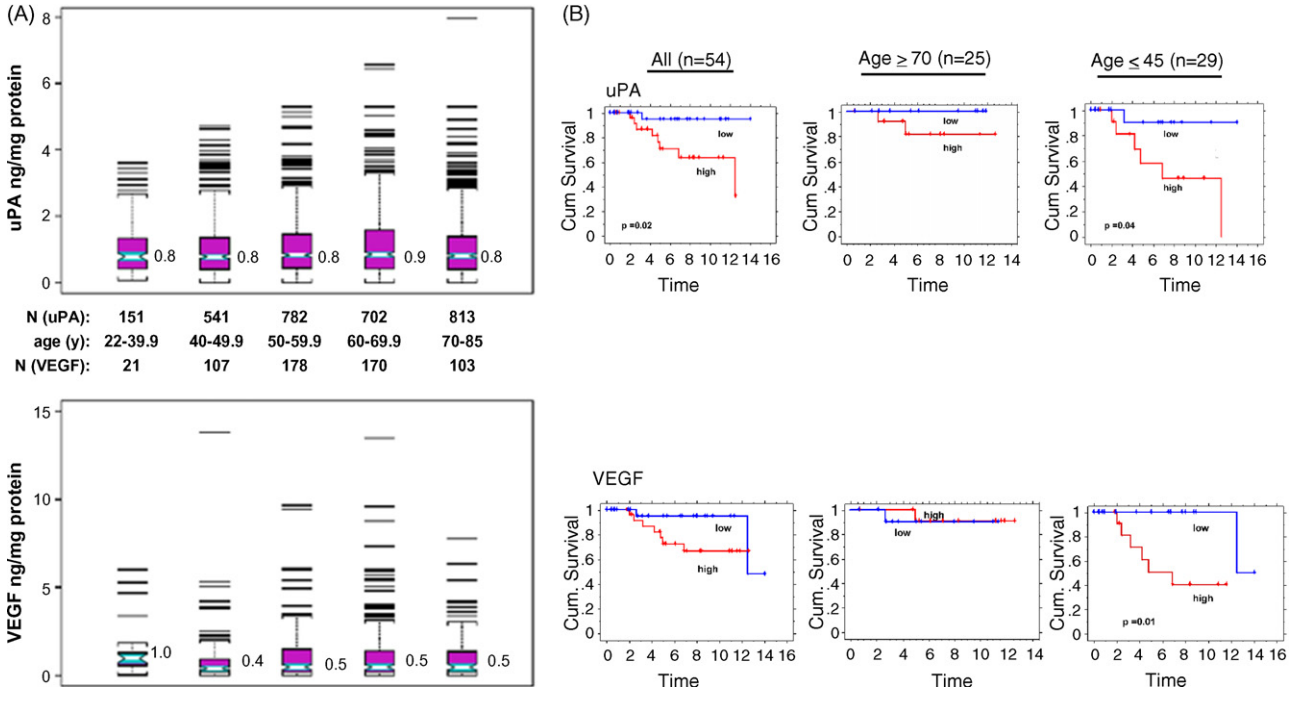

Fig. 4. Age associations for uPA and VEGF protein content from Swiss breast cancer archive (panel A), and Kaplan–Meier relapse-free survival curves based on level of breast cancer uPA and VEGF transcript expression (high, low) from American/UCSF breast cancer archive (panel B). Unselected Swiss samples were assayed as described in Fig. 3 (panel A), with figures modified from previous publication [36]. American/UCSF archive contained 54 node-negative, ER-positive breast cancer cases selected according to late-onset (≥70 years, $n = 25$) or early-onset (≤45 years, $n = 29$). Dichotomization for uPA and VEGF expression levels (high, low) was based on mean-centered transcript values, measured as previously described; figures were modified from previous publication [37]. Significant differences between the cumulative survival curves were determined by Log Rank analyses (only p values <0.05 shown).

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Table 1
Frequency of wildtype p53 (p53wt) versus mutated p53 (p53mut) found in ER-positive (ERpos) and ER-negative (ERneg) subsets of early-onset and late-onset breast cancers

$n = 289$	ERneg/p53wt (%)	ERpos/p53wt (%)	ERneg/p53mut (%)	ERpos/p53mut (%)
Late-onset (≥70 years), $n = 154$	25 (16.2%)	107 (69.5%)	12 (7.8%)	10 (6.5%)
Early-onset (≤45 years), $n = 135$	49 (36.3%)	64 (47.4%)	14 (10.4%)	8 (5.9%)

$p = 0.0004$, Fisher Exact.

different clinical outcomes (relapse-free survival) when comparing late-onset and early-onset breast cancers of similar type [37]. As shown in Fig. 4 (panel B), when transcript levels of VEGF and uPA were assessed in a different population of late-onset (≥70 years, $n = 25$) and early-onset (≤45 years, $n = 29$) node-negative ER-positive breast cancer cases, higher levels of either VEGF or uPA expression were associated with significantly more relapses in the early-onset cases but were not as prognostic in the late-onset cases, despite comparable expression levels of VEGF and uPA in both age cohorts. It will be important to discern if such age-specific outcome differences, in the absence of intrinsic differences in prognostic biomarker tumor expression levels, can be confirmed in future studies. If so, such observations would point to important age-specific differences in clinical susceptibility to biologically similar breast tumors.

5.3. *Early-onset and late-onset breast cancers arise by epigenetically different mechanisms*

The strong inverse age relationships observed between breast cancer ER content or positivity and the multiple indices reflecting breast cancer growth and genetic instability raised concerns about the relative importance of age versus ER status in determining breast cancer biology. Several prospective studies were recently initiated to address this issue, using early-onset (≤45 years) and late-onset (≥70 years) breast cancer specimens of known ER status, derived from two independent breast tumor cryobanks (American/UCSF, Italian). DNA extracted from these samples was analyzed for p53 mutations (exons 5–8) and whole genome aberrations by array comparative genomic hybridization (CGH), while RNA extracted from these samples was analyzed by high-throughput expression microarrays, performed as previously described [23,38]. Table 1 shows the frequency of wild-type p53 (p53wt) versus mutated p53 (p53mut) found in ER-positive and ER-negative subsets of early-onset ($n = 135$) and late-onset ($n = 154$) breast cancers, regardless of tumor stage. The most significant differences to be noted are that late-onset breast cancers are 1.5-fold more likely to be ER-positive/p53wt and 0.45-fold as likely to be ER-negative/p53wt as compared to early-onset breast cancers. While p53 mutations are much less frequently found in ER-positive as compared to ER-negative 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 [39]. Likewise, when array CGH changes were compared between 27 early-onset and 44 late-onset ER-positive ductal breast cancer cases, the two most commonly observed ER-positive breast cancer genotypes (1q gain/16q loss and amplifier genotypes) were equally represented in both age cohorts [23,40]; and no significant age differences were apparent in any of the observed genome-wide aberrations, including frequencies of the most common breast cancer amplicons (e.g. *ERBB2* amplicon: 11% in early-onset, 5% in late-onset). In contrast, when expression microarray changes were compared between 53 early-onset and 48 late-onset ER-positive node-negative breast cancer cases, both unsupervised and supervised analyses of the 5.1K variably expressed genes identified significant age-specific differences [40]. Unsupervised analysis revealed that ER-positive breast cancers are heterogeneous and comprise as many as six different transcriptome subtypes including two with a significant age bias. Supervised analyses revealed that late-onset ER-positive breast cancers express significantly higher levels of ER transcripts as compared to early-onset ER-positive cases; increased levels of some tumor suppressors, developmental regulators, and apoptosis inducers; and decreased levels of specific growth regulators and mitotic factors. These findings provide a new mechanistic basis for claiming that when ER status is controlled for, early-onset breast cancers exhibit much greater proliferative potential than late-onset breast cancers, potentially explaining in part their earlier clinical appearance [40]. While it is surprising that early-onset breast cancers appear to lack significant genomic differences from late-onset breast cancers, there appear to be sufficient epigenetic/transcriptome differences to conclude that when ER status is controlled for, late-onset and early-onset breast cancers arise by fundamentally different biological mechanisms [40]. Other age cohort studies of this design and type are now needed to further generalize about potential age-related biological differences driving ER-negative breast tumorigenesis, as well as the many other age-associated epithelial malignancies besides breast cancer.

6. Conclusions

Whether the molecular and cellular effects of normal mammary gland aging produce background effects from which breast malignancy must be differentiated or in some way contribute to the breast carcinogenic process remains a question of fundamental importance. Clinical observations and biomarker studies indicate that late-onset breast cancers grow more slowly and are biologically less aggres-

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sive than early-onset breast cancers, even when controlled for ER receptor expression, supporting the conclusion that the biology of breast cancer is age-dependent. Initial studies comparing early-onset and late-onset ER-positive breast cancers for DNA mutations and whole genome aberrations as well as RNA transcriptome differences suggest that epigenetic changes rather than genotypic variation account for most of the age-dependent biological and clinical differences observed in hormone-dependent breast cancer.

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Acknowledgements

The author wishes to expresses sincere appreciation to his many coauthors who contributed to the original studies discussed herein; particularly noteworthy are his longstanding collaborations with Ann D. Thor, Dan H. Moore, Joe W. Gray, Donna Albertson, Serenella Eppenberger-Castori, and Urs Eppenberger. Outstanding contributions by investigators-in-training, Vita Fedele and Christina Yau, were critical to furthering our understanding of this important area of breast cancer research. Lastly, the comments of Judy Campisi, Ph.D., were most helpful in the preparation of this manuscript. The author's studies were supported in part by National Institute of Health grants R01-AG020521, R01-CA71468, P01-AG025901, and P50-CA58207; California Breast Cancer Research Program grant 10YB-0125; and Hazel P. Munroe memorial funding to the Buck Institute.

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Biography

Christopher C. Benz, M.D., Director of Buck Institute Program on Cancer and Developmental Therapeutics, and adjunct professor of medicine in UCSF Division of Hematology-Oncology and Comprehensive Cancer Center, earned his B.S. in biochemistry at University of California, Los Angeles (UCLA) in 1968, and M.D. at University of Michigan School of Medicine (Ann Arbor) in 1972. He completed his internal medicine and oncology specialty training at the Vancouver General Hospital, University of British Columbia in 1978, and then finished his postdoctoral training and joined the medical faculty at Yale University School of Medicine. In 1983 he was recruited to join the Division of Hematology-Oncology (Department of Medicine) and Cancer Research Institute of the University of California, San Francisco (UCSF) and, shortly thereafter, also became a member of the Joint UCSF/UC Berkeley Bioengineering Graduate Program. In 2000, Dr. Benz relocated his 20-year-old federally funded UCSF breast cancer research program to become one of the founding faculty of the newly opened Buck Institute for Age Research in Novato, CA. He continues to maintain his professorship at UCSF, cares for patients at the UCSF/Mt. Zion Breast Care Clinic, and plays an active role as senior member of the UCSF Comprehensive Cancer Center's Breast Oncology Program.

As Director of the Buck Institute's Program on Cancer and Developmental Therapeutics, Dr. Benz's translational research program focuses on identifying molecular strategies to improve breast cancer diagnostics and therapeutics, with a special emphasis on trying to understand and interrupt the link between breast cancer and aging. He has published nearly 200 peer-reviewed manuscripts and serves on multiple national and international review and oversight committees, including the National Cancer Institute's DTP/DCTD Biological Resources Branch Oversight Committee and the American Association of Cancer Research's Task Force on Cancer and Aging.

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