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COMMENTARY

Breast cancer and amyloid bodies: is there a role for amyloidosis in cancer-cell dormancy?

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Abstract: Breast cancer and Alzheimer's disease (AD) are major causes of death in older women. Interestingly, breast cancer occurs less frequently in AD patients than in the general population. Amyloidosis, the aggregation of amyloid proteins to form amyloid bodies, plays a central role in the pathogenesis of AD and other human neuropathies by forming intracellular fibrillary proteins. Contrary to popular belief, amyloidosis is a common occurrence in mammalian cells, and has recently been reported to be a natural physiological process in response to environmental stress stimulations (such as pH and temperature extremes, hypoxia, and oxidative stress). Many proteins contain an intrinsic "amyloid-converting motif", which acts in conjunction with a specific noncoding RNA to induce formation of proteinaceous amyloid bodies that are stored in intracellular bundles. In cancer cells such as breast and prostate, the process of amyloidosis induces cells to enter a dormant or resting stage devoid of cell division and proliferation. Therefore, cancer cells undergo growth cessation and enter a dormant stage following amyloidosis in the cell; this is akin to giving the cell AD to cease growth.

Keywords: α-fetoprotein, noncoding RNA, amyloid bodies, dormancy, breast cancer, Alzheimer's disease

Introduction

Breast cancer is a disease of aging in women aged 65 years and older, with five times the incidence of this disease in younger women.[1] Alzheimer's disease (AD) is the sixth-leading cause of death in the US, with a current prevalence of 5.4 million Americans.[2] Recent studies have shown that women are at greater risk of AD than men.[3] However, the incidence of breast cancer in female AD patients appears to be lower than in the general population. Furthermore, it appears that women survivors of breast malignancy rarely develop AD simultaneously with cancer.[4–6] A meta-analysis of 50 observational studies found that the presence of AD was associated with reduced co-occurrence of breast cancer.[7] In fact, AD was more common than breast cancer among older women. A report by Zhao et al[8] also suggested that AD patients appear to be at lower risk of breast cancer occurrence.

Although less likely to develop breast neoplasms, female patients with AD co-occurring with breast tumors may be less likely to experience therapeutic treatments. Patients with AD and breast cancer may be more likely to experience deficits in treatment. Gorin et al found that female patients with AD received less treatment for breast cancer than comparable females without AD.[2] Another study found that chemotherapy and radiation were administered to female breast cancer patients with AD less frequently than to non-AD female patients.[9] Also, patients with AD had a lower likelihood of cancer-related surgery than patients without AD.

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Although the inverse relationship between breast cancer and AD in women has been documented, the reasons for this relationship have not been fully explored. Many studies have examined amyloidosis (Ald), the process creating the toxic plaque deposits in the brain (amyloids) that characterize AD. The formation of plaques in AD is known to be a result of overabundant amyloid-protein aggregates stored in brain cells. These stored protein bundles of amyloid bodies (A-bodies) clog brain-cell activities and signal-transduction pathways, inducing a state of cell dormancy. Interestingly, malignant breast and other tumors can undergo similar A-body aggregations that also lead to a condition of cell dormancy. The present paper describes recent additional findings that focus on how Ald acts on cancer cells to retard their growth. In addition, this report addresses how A-bodies are produced and describes the characteristics and traits of the amyloid-forming triggers. Finally, the Ald process is discussed in lieu of recent in vitro and in vivo cancer-cell studies demonstrating that the presence of the nonproliferating dormant state may be a future target for anticancer therapy, at least for a subset of drugs (see "Conclusion" section).

Amyloid bodies

A-bodies are insoluble proteinaceous fibrous aggregates formed from cross-β polymerization of β-pleated sheets.[10] They exhibit birefringence in polarized light and fluorescence when stained with Congo red, thioflavin S, and Amylo-Glo dyes. The aggregation of A-bodies leads to fibril formations (10 nm fibrils) and to toxic plaque deposits in brain cells. Therefore, amyloid proteins play a central role in the pathogenesis of AD,[11] due to the formation and presence of A-bodies resulting from Ald, which occurs in other human neuropathies, such as Parkinson's and Huntington's diseases. All these disorders exhibit an unfolded amyloid-protein structure, rather than the native protein fold, and are subject to the unfolded protein response (UPR) pathway. The UPR is a cellular stress response to misfolded or unfolded proteins that are associated with the heat-shock and glucose-shock proteins found in the endoplasmic reticulum; this response is conserved in mammals, yeast, and worms.[12,13]

Amyloidosis

Systemic Ald can appear in two major forms in the body – an Ig light-chain form and a serum amyloid-A form – causing amyloid deposition in such tissues as the kidney, heart, and liver.[14] While the Ig light-chain form is derived from Ig light chains secreted by bone marrow or lymphoid plasma cells, the amyloid-A type originates from serum amyloid-A protein secreted from the liver as an acute-phase inflammatory protein.[15] Examples of systemic Ald links to cancer are observed in 1) the secretion of Ig light chains by myeloma (plasma cell) tumors, and 2) amyloid light-chain deposition in kidneys and early-stage non-small-cell lung adenocarcinomas.[16] In the former instance, myeloma-derived light-chain deposition in the kidney results in paraproteinemia; in the latter case, amyloid accumulation in lung cancer is a consequence of the binding of amyloid fibril to overexpressed amyloid-binding (RAGE) receptors present in lung tumors.[17]

Ald is a by-product of polypeptide assembly, and A-bodies are a highly organized form of protein aggregation that convert native-fold proteins into β-sheet-rich aggregates that are protease K and sodium dodecyl sulfate boiling point-resistant multimers. Interestingly, A-body peptides are amphiphilic, can bind various metals, such as ion, zinc, copper, and cobalt, and can function as cell-membrane disrupters.[18–21] These chelated metal proteins not only disrupt cell membranes, but aid in penetrating cell membranes, binding to receptors, and inducing the formation of endocytic vesicles.[18] Metal binding to the amyloid-peptide sequence is due to the positioning of two histidine residues that aid in producing a tetrahedral symmetrical formation.[22] In the physiological state, the resultant protein fibrils are nontoxic; moreover, the formations are reversible by means of chaperone pathways employing HSP70, HSP90, and GRP78 shock-induced disaggregation.

Contrary to popular belief, Ald is a common occurrence in eukaryotic cells.[23] A recent report further elucidated Ald as a natural physiological response in mammalian cells responding to multiple stress stimulations.[24] The Ald process enables cells to aggregate protein as fibrils for storage, thereby facilitating adaptation to cellular stresses. As such, Ald enables cells to store large quantities of fibrillary proteins and enter a dormant or resting state, while still remaining viable during extended periods of stimulation from extracellular stressors. Eukaryotic cells frequently encounter environmental stress factors, such as inflammation, hypoxia, high temperatures, H_2O_2 peroxidation, acidosis, pH extremes, oxidative stresses, and other conditions associated with growth dysregulation. For example, high extracellular and/or cytoplasmic temperatures activate both the heat/glucose shock response and the UPR.[25] Expression of the chaperone proteins (HSP70/90, GRP78) enables the cell to reduce the total cell volume of misfolded proteins by refolding proteins back to their native folded state.[26,27] In a similar fashion, transcription factors activate several genes that respond to hypoxic environments, augmenting oxygen delivery and increasing glucose metabolism during low-oxygen periods.[28,29] Whatever the

environmental stimulus may be, both the stress response and the UPR are designed to aid in 1) restoring cell homeostasis, 2) repairing cell/molecular damage, and 3) sustaining cell viability in times of environmental stress encounters.

Amyloid-converting motif

In the normal physiological response to stress, as just described, cells induce an amyloid state in their cytoplasmic proteins by invoking and activating a discrete peptidic 30–42 stretch of amino acids (AAs) on polypeptide proteins termed the "amyloid-converting motif" (ACM).[24] Studies show that the AA-sequence stretch of the ACM is crucial in converting cell proteins into A-bodies by interacting with nuclear ribosomal intergenic spacer noncoding RNA (rIgS RNA). For example, rIgS RNA interacting with the toxic β-amyloid peptide (1–42 AA-sequence stretch) is directly involved in the plaque formation in AD and exhibits an ACM-like sequence in the process of mediating amyloidogenesis in vivo.[30] Therefore, the ACM comprises peptide sequences derived from proteins and can be divided into two distinct submotifs consisting of an arginine/histidine (R/H)-rich sequence and a highly amyloidogenic AA sequence that binds Congo red, thioflavin, and Amylo-Glo dyes. The latter domain displays AA (single-letter code: X = any AA) di- and tripeptide clusters, such as KXL, LXK, GXG, and GXL/I, as well as $HX_{5-9}H$, the latter of which lies within or adjacent to the R/H-rich areas.[24] Such ACM sequences are found in one to three distinct regions on many diverse proteins, such as CDK1, residues 100–130; HAT1, residues 228–260; HDAC2, residues 1–33; pVHL, residues 104–140; APP, residues 1–42; α_2M, residues 1,314–1,365; ApoE, residues 200–299; and AFP, residues 464–496.[31]

AFP amyloid-converting motif

From the list of ACM-containing proteins just mentioned, the AA sequence found on the AFP polypeptide best exemplifies the characteristics of this motif. The entire AFP polypeptide contains at least one ACM that is positioned on the third domain of AFP at AA 464–499. This 35-AA sequence has been synthesized and chemically purified, exhibiting the sequence LSEDKLLACGEGAADIIIGHLCIRHEMTPVNPGVG, termed the "growth-inhibitory peptide" (GIP).[32] This 35-AA peptide fragment has been reported to display reduced content of helical secondary structure (10%), high content (45%) of β-sheet/β-hairpin turn structure, and a disordered structure of 45%. Therefore, 90% of the GIP is of a nonhelical secondary structure, largely displaying a β-sheet/disordered structure.[33] It can be observed that the GIP sequence contains multiple signature di- and tripeptide sequences, including GXG (GEG; GVG), GXL/I (GHL), KXL (KLL), and $HX_{5-9}H$ (HLCIRH). The latter represents an R/H sequence, which is a hallmark feature of the ACM. Furthermore, the GIP has been demonstrated to bind to the amyloid dye Congo red and to the 8-anilino-naphthalenesulfonic acid dye that represents an unfolded protein or peptide.[34] Interestingly, AFP has recently been shown to be a biomarker of the UPR in human hepatocellular carcinoma cells.[35] Finally, the GIP sequence has been found to bind zinc and cobalt, due to the positioning of the two histidine residues producing the canonical tetrahedral symmetrical formation.[34]

Cancer-cell implications

As outlined herein and in previous published reports, it appears that cells normally possess a posttranslational Ald pathway that rapidly and reversibly converts native-folded proteins into amyloid-like folds for purposes of cell-compacted protein storage. Audas et al demonstrated that a large number of amyloid proteins are stored in dormant cancer cells.[24] However, the chaperone/UPR pathways can disaggregate A-bodies and convert these dormant cancer cells back to actively proliferating tumor cells. However, if the A-bodies in dormant quiescent cancer cells can be prevented from disaggregating, they might remain in that state for indefinite periods of time. Such A-body dormancy research is currently in progress.[24]

A-body deposits and accumulations have been reported to induce a state of cancer-nongrowth dormancy. It appears that cancer cells do not proliferate during the period in which Ald has occurred, a finding that has major implications for oncological therapy/treatment. Proliferating cancer cells undergo large influxes of proteins into their intracellular compartments, in order to satisfy cell-cycle progression and DNA-synthesis/repair demands during growth. Hypoxia and/or acidosis often accompany such events and can trigger A-body activation of the $rIgS_{28}$ RNA-dependent Ald pathway. Induction of A-body-manifested bundles of protein fibrils is prevalent in the cancer-cell microenvironment, due to multiple stress factors, resulting in Ald formation that produces a state of tumor-cell dormancy.[36–38]

Employing the Ald-model concept, Audas et al performed in vitro cell culture and formalin-fixed human patient cancer-cell immunochemistry procedures, together with in vivo mouse-xenograft assays employing cultured human MCF7 breast cancer cells and human prostate cancer PC3 cells. Their findings indicated that both of the cell lines expressed $rIgS_{28}$ RNA.[24] Furthermore, Benz et al had previously shown

that control MCF7 cells (without growth factors) formed only minimal masses of A-bodies in cell culture and in human breast cancer xenografts in host nude mice.[39] However, when $rIgS_{28}$ RNA was inhibited by silencing, the MCF7 cells did not accumulate A-bodies and failed to stain positively for Congo red/Amylo-Glo dyes. Such RNA-silenced xenografts in mice exhibited large tumor masses, which grew in about 4 weeks. Audas et al further observed that the PC3 cells that generally form only moderately sized amyloid bundles formed large tumor masses when $rIgS_{28}$ RNA was inhibited/silenced and ACM interaction ceased.[24] Therefore, silencing of the noncoding RNA prevented formation of amyloid masses, as evidenced by lack of Congo red/Amylo-Glo positive staining of amyloid foci in the tumor cells, allowing subsequent tumor-cell growth to ensue. These data further support the concept of $rIgS_{28}$ RNA-mediating A-body formation inducing cells to enter a state of dormancy, allowing them to maintain viability during periods of extracellular stress stimulations.

Tumor-cell dormancy

The state of cell dormancy (quiescence) is a lag in growth due to a temporary lack of cell-cycle mitogenic signaling.[36,37] Dormant cells remain in a metabolically active but nonproliferative mitotic arrest that is reversible. After radical surgery of a primary tumor, residual disseminated tumor cells can enter a protracted period of dormancy of indeterminate time prior to mounting a metastatic response.[36] In principle, it would be most efficacious to eradicate cancer cells during this premetastatic quiescent period; if not feasible, attempts could be made to sustain dormancy. It is of interest that 62% of all deaths from human breast cancer occur after the 5-year survival mark, when tumor cells have awakened from dormancy.[40] Survival-signaling pathways that determine dormancy include 1) Wnt and Notch, PI3K–Akt, 3) p130 and p27 (KIP1), 4) EGF receptors, 5) mTOR activation of GTPases, 6) apoptosis and autophagy, and 7) the oxidative response pathway.[37] Chemical substances that carry out such survival signaling encompass cyclin D_1, DNA methyltransferase (DNMT1), FoxM1, $TGF\beta_2$, GAS6, BMP4, RAR, and p21 (WAF1).[37] Therefore, in lieu of destroying dormant tumor cells, enhancing and maintaining the survival-signaling pathways of dormancy would pose a most attractive and effective therapeutic modality.

Conclusion

Multiple studies support the finding that occurrence of AD in older women correlates with reduced presence of breast cancer. One explanation for the reduced occurrence of AD in breast cancer patients might be attributed to the accumulated presence of A-bodies in the breast cancer cells. As described herein, studies employing both human in vitro cell cultures and in vivo mouse xenografts have demonstrated that activation of Ald in breast cancer cells converts the cancer cell into nonreplicating dormancy. The presence of an ACM residing on the targeted protein in combination with a specific nuclear RNA are both required to induce the process of amyloidogenesis. Once assembled, the A-bodies produce bundles of fibrillary proteins that are stored within the cells. The presence of large amounts of stored A-bodies then further promotes the nongrowth state of dormancy. Such dormant cancer cells become ideal targets for a subset of inhibitory therapeutic anticancer drugs, such as the recently described inhibitory RNA miR222/223.[41] Future studies exploring this possibility of inducing AD in cancer cells could contribute invaluable information to the field of oncology.

Disclosure

The author reports no conflicts of interest in this work.

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