Accelerated aging in the tumor microenvironment

Connecting aging, inflammation and cancer metabolism with personalized medicine

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Cancer is thought to be a disease associated with aging. Interestingly, normal aging is driven by the production of ROS and mitochondrial oxidative stress, resulting in the cumulative accumulation of DNA damage. Here, we discuss how ROS signaling, NFκB- and HIF1-activation in the tumor microenvironment induces a form of "accelerated aging," which leads to stromal inflammation and changes in cancer cell metabolism. Thus, we present a unified model where aging (ROS), inflammation (NFκB) and cancer metabolism (HIF1), act as co-conspirators to drive autophagy ("self-eating") in the tumor stroma. Then, autophagy in the tumor stroma provides high-energy "fuel" and the necessary chemical building blocks, for accelerated tumor growth and metastasis. Stromal ROS production acts as a "mutagenic motor" and allows cancer cells to buffer—at a distance—exactly how much of a mutagenic stimulus they receive, further driving tumor cell selection and evolution. Surviving cancer cells would be selected for the ability to induce ROS more effectively in stromal fibroblasts, so they could extract more nutrients from the stroma via autophagy. If lethal cancer is a disease of "accelerated host aging" in the tumor stroma, then cancer patients may benefit from therapy with powerful antioxidants. Antioxidant therapy should block the resulting DNA damage, and halt autophagy in the tumor stroma, effectively "cutting off the fuel supply" for cancer cells. These findings have important new implications for personalized cancer medicine, as they link aging, inflammation and cancer metabolism with novel strategies for more effective cancer diagnostics and therapeutics.

One way to think about cancer is as a disease associated with normal aging.[1,2] During aging, mitochondrial ROS production results in DNA damage and mitochondrial dys-function.[3-5] Interestingly, we see that cancer cells can also induce a form of "accelerated aging" in normal adjacent fibroblasts, via ROS production and oxidative stress.[6-15]

Oxidative stress in cancer-associated fibroblasts then results in the activation of two major transcription factors, namely NFκB and HIF1.[10] NFκB is the master regulator of the innate immune response (inflammation), while HIF1 drives metabolic re-programming with the onset of aerobic glycolysis (the "Warburg effect").[16,17] Both NFκB and HIF1 are also key activators of autophagy and mitophagy.[12] Thus, "accelerated aging" via ROS production drives inflammation, cancer metabolism and autophagy (**Fig. 1**). This, in turn, results in DNA damage in cancer cells (driving genomic instability) and fuels oxidative mitochondrial metabolism in cancer cells (via the stromal production of high-energy metabolites, such as lactate, ketones and glutamine).[10,12,13] Ketones and lactate, in turn, stimulate tumor growth and metastasis.[18] A loss
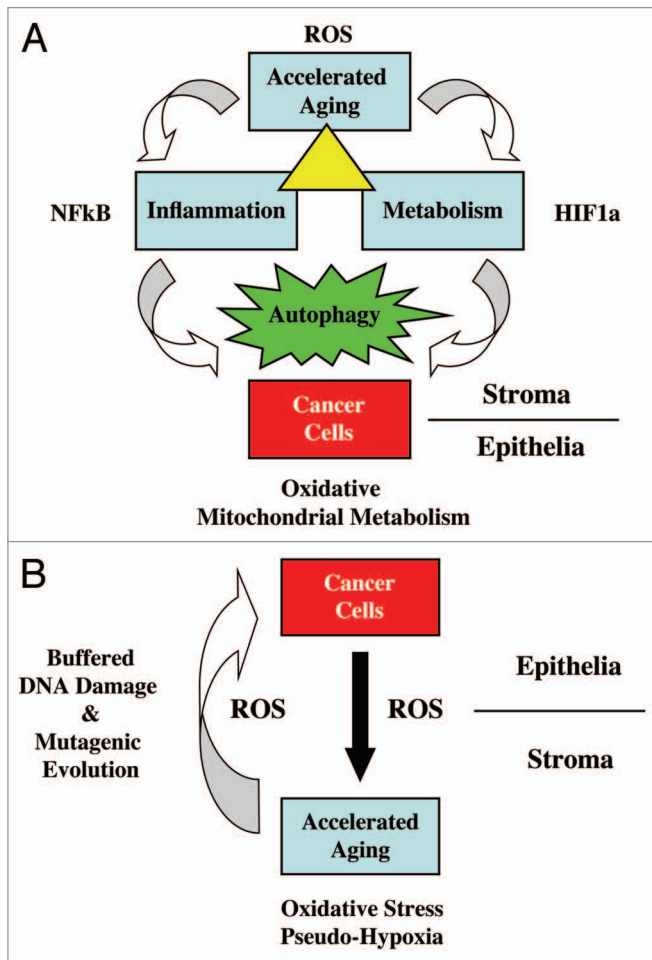
Figure 1. Accelerated aging drives DNA damage, inflammation, aerobic glycolysis and autophagy in the tumor microenvironment, fueling tumor progression and metastasis. See text for further details. (A) Cancer cells induce ROS production (accelerated aging) in adjacent normal fibroblasts, which results in NFκB-activation (inflammation) and HIF1-stabilization (cancer metabolism). Their combined effect drives autophagy in the tumor stroma. Stromal autophagy, via the production of high-energy nutrients (lactate, ketones and glutamine), stimulates oxidative mitochondrial metabolism in cancer cells. (B) Accelerated aging in fibroblasts produces ROS that induces DNA damage in fibroblasts and adjacent cancer cells (via a Bystander effect), fostering tumor-stroma co-evolution.

of stromal caveolin-1 (Cav-1) provides a robust biomarker for this "accelerated aging" phenotype in the tumor stroma, and is functionally associated with oxidative stress, inflammation, aerobic glycolysis and autophagy.[15]

Four recent papers published in the journal *Cell Cycle* further validate this model of cancer as "accelerated host aging."

The first paper establishes that stromal fibroblasts and cancer cells are metabolically coupled, via a lactate shuttle that transfers lactate from glycolytic cancer-associated fibroblasts to oxidative cancer cells, via the compartmentalization of MCT transporters.[19] This is accomplished, in part, by the upregulation of MCT4 expression in cancer-associated fibroblasts, via oxidative stress.[19] Thus, aggressive cancer cells use lactate as a fuel source, via oxidative mitochondrial metabolism.

The second paper shows that oxidative stress in cancer-associated fibroblasts results in a "cytokine storm," via NFκB-activation.[20] Individually, each of these inflammatory cytokines was also sufficient to induce autophagy in stromal fibroblasts.[20] This observation explains how inflammation literally "fuels" tumor growth, via stromal autophagy.[20]

The third paper analyzes the transcriptional profiles of laser-captured tumor stroma, associated with a lethal tumor microenvironment in human breast cancer patient samples.[21] In this paper, a Cav-1-deficient tumor microenvironment shows the same patterns of gene expression as those normally associated with aging, Alzheimer disease, DNA damage, oxidative stress, inflammation, hypoxia, HIF1- and NFκB-activation (**Fig. 2**).[21] In fact, the Alzheimer disease brain signature can identify which breast cancer patients will develop metastasis.[7,8]

Finally, the fourth paper shows that when breast cancer cells (MCF7) use lactate as a "fuel supply," they undergo mitochondrial biogenesis[10,12] and transcriptionally they appear similar to "stem cells."[22] These lactate-induced gene signatures predict recurrence, metastasis, and poor overall survival in breast cancer patients, directly linking oxidative mitochondrial metabolism with clinical outcome.[22] These observations provide a new starting point for personalized cancer medicine, linked to cancer metabolism, termed "metabolo-genomics."[22] Interestingly, administration of lactate to a human breast cancer xenograft model (MDA-MB-231 cells) increases the rate of lung metastasis >10-fold.[18]

In accordance with this "accelerated host aging" model, a loss of Cav-1 in the tumor stroma is a marker of oxidative stress and inflammation in the tumor microenvironment,[7,8,10,23,24] and is associated with metastasis and poor prognosis in breast and prostate cancer patients.[24-28] Similarly, in mouse animal models, a loss of Cav-1 is associated with accelerated aging,[29] and an increased susceptibility towards various oncogenic stimuli,[30-34] as well as oxidative stress, DNA damage and autophagy.[7,10] Thus, "accelerated host aging" provides a "fertile soil" for tumor growth and metastasis.

This new model of cancer as "accelerated host aging" suggests that we should be treating cancer patients with powerful antioxidants. Interestingly, "catalase therapy" in pre-clinical models efficiently blocks both tumor recurrence and metastasis.[35-42] Genetic reductions in mitochondrial oxidative stress (via transgenic overexpression of catalase) reduces
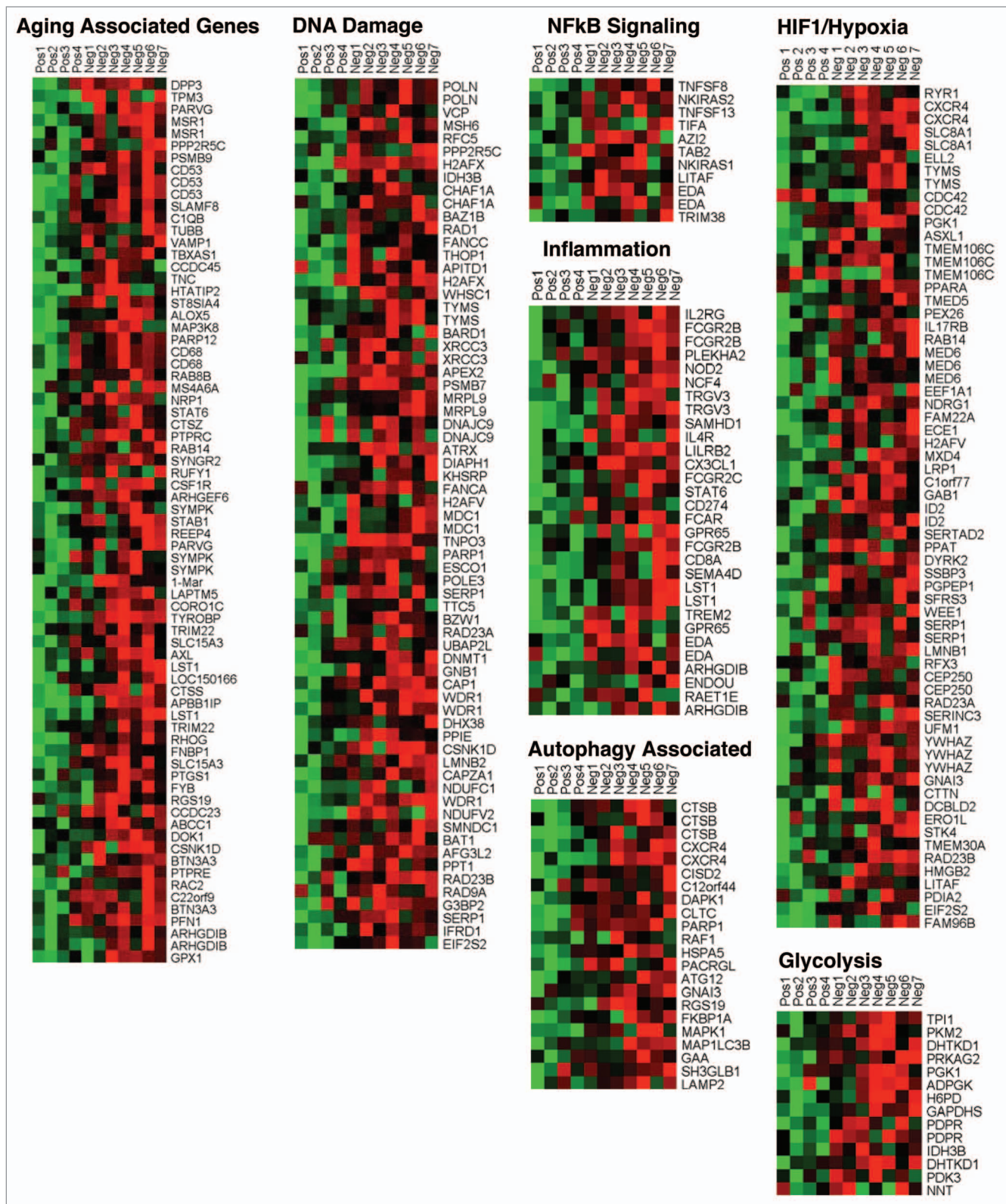
Figure 2. A loss of Cav-1 in the tumor stroma of human breast cancer patients is associated with aging, DNA damage, inflammation, cancer metabolism and autophagy. The transcriptional profiles of Cav-1-positive (+) tumor stroma (n = 4) versus Cav-1-negative tumor stroma (n = 7) were compared, via laser-capture microdissection. Note that Cav-1-deficient stroma shows the upregulation of aging (73 transcripts), the DNA damage response (67 transcripts), NFκB signaling (11 transcripts), the immune response (31 transcripts), HIF1/hypoxia target genes (65 transcripts), glycolysis/pyruvate metabolism (15 transcripts) and autophagy (22 transcripts). Modified and reproduced with permission from reference 21. In human breast cancer patients, a loss of stromal Cav-1 is associated with early tumor recurrence, lymph-node metastasis, drug-resistance and overall poor survival, conferring a "lethal" tumor microenvironment.[24-28]

metastatic tumor burden in MMTV-PyMT mice by ~13-fold.[43,44] Similarly, treatment of breast cancer patients with antioxidants (Vitamins C and E), significantly reduces both mortality and recurrence, and this protective effect is nearly doubled if the patients did not undergo radiation therapy.[45] Radiation therapy is known to increase oxidative stress and DNA damage, thereby negating the positive effects of antioxidants.[45]

Successful antioxidant therapy should prevent DNA damage, stopping cancer cell evolution and halting autophagy in the tumor stroma, effectively "starving" cancer cells. Thus, we should re-consider using antioxidants (N-acetyl-cysteine and Vitamins C/E), mitochondrial poisons (metformin) and autophagy inhibitors (chloroquine and hydroxy-chloroquine), as potential anti-cancer agents.

Unfortunately, most current cancer therapies are toxic and work by increasing oxidative stress and DNA damage, thereby "accelerating host aging." In fact, most current therapies (chemo-therapeutics and radiation) also increase a patient's risk for secondary malignancies, such as leukemia or lymphoma.

As a consequence, we should carefully re-think how to more effectively treat cancer patients, without increasing oxidative stress and "accelerated aging."

Aging studies in flies and mice also point towards a causative role for inflammation in reduced life span.[46,47] For example, flies selected for increased longevity show the specific downregulation of gene transcripts normally associated with inflammation and the immune response.[47] Similar results were obtained in mice, where they observed that gene transcripts associated with cytokine production, the immune response and inflammation were specifically upregulated during aging.[46] Interestingly, caloric restriction specifically reduced the expression of these aging-associated inflammation genes, but not other aging-associated gene transcripts.[46] Thus, there appears to be a direct metabolic link between aging and inflammation. We believe this metabolic link is that inflammation induces systemic increases

in autophagy/mitophagy and aerobic glycolysis.[20] Thus, "cutting off the fuel supply" via caloric restriction decreases inflammation, which is promoting aging and cancer via increased ROS production and the ensuing DNA damage.

In further support of this notion, N-acetyl-cysteine (NAC), which functions as a powerful antioxidant and NFκB-inhibitor, increases lifespan in flies and in mice by >25%.[48-50] NAC is also protective against tumor formation.[51,52] Thus, exploiting the dietary use of NAC and other antioxidants (metformin) or autophagy inhibitors (hydroxy-chloroquine) may be helpful in preventing "accelerated host aging" in the tumor microenvironment.[10,11]

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