REVIEW

A Mitochondrial view of aging, reactive oxygen species and metastatic cancer

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Summary

This perspective article highlights the growing evidence placing mitochondria and mitochondrial function at the center of cancer as an age-related disease. The discussion starts from the mitochondrial free radical hypothesis that predicts the involvement of endogenous mitochondrial reactive oxygen species (ROS) in cancer development and summarizes studies demonstrating the impact of the modulation of ROS levels on cancer development and metastasis. Cancer is fundamentally a complex interplay of cell growth, division, metastasis and death-processes connected to mitochondria through energy metabolism. Based on this evidence, therapeutics focused on mitochondrial function and mitochondrial ROS production are an attractive approach to modulating the progression of metastatic cancer and the general improvement of human health span.

Key words: mitochondria; cancer; ROS; aging; senescence.

Metastatic cancer is an ROS-mediated disease of aging

Longevity measurements have been applied with spectacular success in studies of invertebrate aging. However, application to mammals, including mice and humans, is profoundly limited by their comparatively long lifespans and the associated costs of accumulating life table data. Nevertheless, there are strong indications that modulation of mammalian aging can be successful. Our own studies of extended longevity in mice that overexpress mitochondrial-targeted catalase (mCAT) serve as an example (Schriner et al., 2005). The limitations of lifespan as an endpoint in these types of studies and the uncertain correlations of lifespan with health parameters have led biogerontologists to give increasing attention to conditions of improved ‘health span’, i.e. enhanced health and organ function during aging and protection from age-related diseases such as cancer (Butler et al., 2008). The highest risk factor for developing most types of cancer is advancing age (Jemal et al., 2006). In this regard, we have documented an improved health span in mCAT mice by the demonstration of a decreased incidence of a range of cancers during aging (Treuting et al., 2008). A significant finding was decreased malignancy and tumor burden in tissues of epithelial origin, suggesting that mCAT was suppressing progression but not necessarily initiation of age-related cancer. Other studies in transgenic mice expressing antioxidant enzymes have not found any association with cancer and aging. For example, over expression of CuZnSOD, MnSOD, or GPX4 showed no effect on lifespan and little effect on tumor incidence (Pérez et al., 2009), which would appear to conflict with our hypothesis that metastatic cancer is associated with antioxidant enzyme function in an age-related manner. However, it may be that the over expression of these genes affects responsive signaling pathways in different ways and/or in different cell types. Further studies are needed to address the biologic and clinical implications of these apparently conflicting data.

Regardless of the antioxidant molecular events, metastases to distant organs are the unfortunate consequences of most late-stage human cancers and present intense treatment challenges in the elderly. Recent evidence now suggests that metastasis may be driven by reactive oxygen species (ROS) (Ishikawa et al., 2008) used cytoplasmic hybrid technology to replace the endogenous mtDNA in a mouse tumor cell line that was poorly metastatic with mtDNA from a cell line that was highly metastatic. They found that the low metastatic recipient tumors acquired the metastatic potential of the high metastatic transferred mtDNA, which contained mutations in the gene encoding NADH resulting in a deficiency in respiratory complex 1 activity and over production of ROS. Pretreatment of the highly metastatic cells with ROS scavengers reduced the metastatic potential in mice. A decline in mitochondrial energy production with age can generate increased ROS, which cause mitochondrial mutations and additional ROS production (Nemoto et al., 2000; Valko et al., 2004). It has recently been reported that mitochondrial polymorphisms play a role in women’s risk for breast cancer suggesting an association with increased ROS production (Bai et al., 2007). We have indirect unpublished evidence that this is substantiated in a mouse model of metastatic breast cancer. When PyMT transgenic mice (Lin et al., 2003) were crossed with transgenic mice expressing mCAT (Schriner et al., 2005), they showed a robust reduction in metastatic tumor burden in the lungs, suggesting...
that ROS may be driving metastatic tumor progression in this mouse model (Ladiges, manuscript in preparation). The metastatic cells may be altering ROS signaling by constitutively activating mitogenic and metabolic pathways that further increase the levels of endogenous ROS. Rapid tumor growth results in hypoxia and glucose deprivation, which stimulates the production of chemo-attractants and the recruitment of ROS-producing macrophages (Sica et al., 2002). These polarized macrophages could be programmed for tumor support, because their deletion in a mouse model of metastatic breast cancer prevents the metastatic phenotype (Lin et al., 2007). Therefore, metastatic cancer is highly dependent on a friendly and actively supporting microenvironment for its invasive qualities.

A long-term goal of the genetics of aging must be the translation of basic research findings to the development of pharmacologic interventions for improved human health span. In the case of solid human tumors, it takes some 20 years from the time of exposure to a carcinogen to the clinical appearance of a tumor. Thus, therapies directed at the delay of tumor progression should be an important component of age-related research in cancer. It is generally apparent that in most cases where mitochondrial dysfunction contributes to disease, a major cause of damage is ROS produced by mitochondria, either directly or as a secondary disruption of other cellular metabolic pathways. This evidence supports the mitochondrial free radical theory of aging and assumes that the accumulation of oxidation-induced damage of macromolecules, including DNA, is associated with aging. It follows that ROS contribute to the age-related development of cancer, although the cellular and carcinogenic mechanisms are not well understood. Based on this concept, we suggest that relevant strategies for treating metastatic cancer can arise by decreasing mitochondrial oxidative damage and altering ROS levels as the result of increased amounts of endogenous mitochondrial antioxidant enzymes or by ectopic delivery of antioxidant enzymes or drugs to mitochondria. A focus on mitochondrially targeted antioxidants rather than conventional, untargeted antioxidants is warranted given the general failure of systemic antioxidants (e.g. vitamin E) to retard aging and age-related diseases including cancer (Lonn et al., 2005).

**Mitochondrial ROS are signals for senescence and apoptosis**

Altered mitochondrial function is implicated in a wide range of primarily age-related diseases (Wallace, 2005). Numerous mechanisms may link mitochondrial dysfunction to these diseases, and the most prominent of these may be the mitochondrial production of ROS. However, ROS are a natural byproduct of normal mitochondrial function. Mitochondrial metabolism leads to the formation of the superoxide radical, the first molecule in the pathway responsible for the production of ROS (Fig. 1). Reactive oxygen species generation occurs as the result of chronic leakage of electrons during normal respiratory function or from bursts of intra-mitochondrial, cytoplasmic, or extracellular ROS production in response to stress or inflammation. Within the mitochondria, superoxide is converted to diffusible hydrogen peroxide and subsequently to the highly mutagenic and toxic hydroxyl radical. Mitochondrial ROS production became the key tenet of the mitochondrial free radical hypothesis of aging. ROS-mediated macromolecular damage, if not repaired, can cause the progressive failure of cellular machinery, organ aging and the onset of age-related disease. Thus, mitochondrial oxidative stress is an attractive mechanism to explain age-dependent oncogenesis and metastatic tumor progression.

A hallmark of cancer is a disrupted balance of cell growth, cell division and cell death. Not surprisingly, mitochondria have an intimate role in all of these processes (Fig. 2). Senescence dictates arrest of cellular growth and thus resistance to cancer. There is growing evidence of a causal link between cancer, senescence, ROS and mitochondria. Early studies demonstrated the acquisition of senescence-like growth arrest in fibroblasts exposed to increased ambient oxygen and hydrogen peroxide (Passos & von Zglinicki, 2005). This led to additional studies which demonstrated that in vitro senescence is associated with high levels of endogenous ROS, increased levels of protein

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*[Image 307x554 to 534x720]*

**Fig. 1** Mitochondrial-mediated oxidative stress and DNA damage. Electron leakage leads to superoxide production (O$_2^-$), which is converted to hydrogen peroxide (H$_2$O$_2$). The highly reactive hydroxyl radical (OH) is generated by the Fenton reaction unless H$_2$O$_2$ is reduced by antioxidants such as glutathione peroxidase.

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*[Image 463]*

**Fig. 2** Central role of mitochondria in mediating senescence or cancer during aging. Increased reactive oxygen species accumulation as the result of endogenous or exogenous insults can cause mitochondrial dysfunction and subsequent triggering of apoptosis, senescence and/or promotion of metastatic tumor development.
amounts of H2O2 are being generated and the presence of catalase or catalase mimetics will prevent apoptosis in cancer cells. However, in nontumor cells in the microenvironment, the presence of catalase may be neutralizing H2O2, preventing cytokine signaling responsible for metastatic tumor progression.

**Treating metastatic disease by altering mitochondrial ROS production**

Treatment of metastatic disease has an alarmingly high rate of failure because effective targets still have not been identified. Inherent or acquired chemotherapeutic resistance and dose-limiting toxicity limit many agents used in the treatment of metastasis. A number of mouse cancer models exhibit metastases and are readily amenable for studies of involved factors and therapeutic interventions. The use of novel approaches in these types of mouse cancer models may uncover the mechanisms, including the role of mitochondria, in organ-specific metastasis, an important question for human tumor biology. In addition, an important goal in the treatment of metastatic disease is the development of novel, nontoxic therapeutic agents.

Generally, it is considered that cellular senescence can result in the suppression of tumor progression. In this regard, the tumor microenvironment may play a major role in determining whether tumor cells are allowed to progress. It may be that with aging the microenvironmental changes in response to an aggressive tumor are inadequate or maladaptive and allow metastasis. Less aggressive tumors may send different signals that trigger apoptosis and help to suppress tumor progression. Campisi’s group has evidence to suggest that soluble factors released by the hypersecretory senescent cells that accumulate in the stroma of aging tissues can facilitate tumor progression via the diffusion of mitogens and the alteration of extracellular matrix via the enzymatic actions of collagenases and elastases (Coppe et al., 2008). These nontumor cells may be attractive targets for strategies to reverse ROS-mediated senescence using mitochondrial-targeted antioxidants.

While awaiting definitive demonstration of the relationships between ROS and cancer development, a number of groups are assessing the biologic impact of modulating ROS. Our group is focusing on delaying or preventing metastasis and metastatic progression because we have shown that transgenic mice expressing mCAT have decreased age-associated malignancies and metastatic tumor burden (Treuting et al., 2008). Our approach is based on the concept that attenuation of ROS in nontumor cells of the microenvironment is an effective means of counteracting the pro-tumor support that these cells are being programmed by cancer cells to provide. Several strategies could be considered. It is conceivable that with improved viral vector systems, catalase directed specifically for mitochondria could be inserted directly into relevant tissues. For example, if the distant site of metastasis is the lungs, delivery systems to the lungs could be tested. A second strategy would be the use of antioxidant mimetics, which are small molecules that have an affinity for mitochondria or are conjugated to a carrier that has an affinity for mitochondria (Murphy & Smith, 2007). The real question is whether they act in an anti-oxidant or pro-oxidant manner, as the vitamin E analog, alpha-tocopherol, has been shown to induce cancer cell death by a process involving mitochondrial-mediated ROS (Ralph & Neuzil, 2009). However, the effect on nontumor cells in the microenvironment is less well understood. It may be that mitochondrial-specific antioxidant mimetics have different effects in malignant tumor cells and nontumor cells, i.e. pro-oxidant and anti-oxidant, respectively, so that the end result would be a complementation of activity to suppress and eliminate metastatic cells.
Conclusions

A central role for mitochondria in cancer and aging is an exciting synthesis of hypotheses and evidence, which connects these closely related phenomena. The prominent involvement of mitochondrial ROS suggests that these byproducts of normal mitochondrial function are likely important players in a very wide range of cancer and age-related processes. Therefore, a better understanding of the precise functions of ROS in pathways such as senescence, apoptosis and macromolecular damage is paramount. Emerging mouse models of modulated ROS, small molecule, targeted antioxidants and surrogate models of various cancers provide a full armamentarium to pinpoint the beneficial and harmful actions of ROS. It is hoped that understanding the larger picture of ROS metabolism, signaling and damage will provide opportunities for therapeutic interventions, which are in critical need to address aging, age-related diseases and metastatic cancer.

References


