Chapter 12

Abnormalities in Growth Control, Telomerase Activity, Apoptosis, and Angiogenesis Linked to Mitochondrial Dysfunction

Hanahan and Weinberg (1, 2) argued that genomic instability was an essential enabling characteristic for manifesting the hallmarks of cancer. However, I suggest that OxPhos insufficiency is the essential enabling characteristic in the origin of cancer. Our recent hypothesis defined how the acquired capabilities of cancer could be linked specifically to impaired energy metabolism (3). Kroemer and Pouyssegur (4) also provided a nice overview on how the Hanahan and Weinberg hallmarks of cancer could be linked to signaling cascades and to the metabolic reprogramming of cancer cells. Although similar topics are considered in the Kroemer/Pouyssegur review and in my treatise, my view differs from theirs on the role of respiratory damage in the origin of cancer. Kroemer and Pouyssegur mentioned that Warburg's respiratory damage hypothesis is not universally applicable to all cancers and cited the study of Funes et al. (5) to support their contention. I consider insufficient respiration as universal phenotype of all cancers. I described in Chapter 7 how none of the studies suggesting normal respiration in cancer cells have excluded amino acid fermentation as an alternative explanation for their findings. Funes and coworkers also did not consider the possibility of mitochondrial amino acid fermentation as a source of tumor cell ATP. I also disagree with the view of

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Kroemer and Pouyssegur that tumor cells have a growth advantage over normal cells. I will address the growth advantage topic more in Chapter 17.

GROWTH SIGNALING ABNORMALITIES AND LIMITLESS REPLICATIVE POTENTIAL

A central concept in linking abnormalities of growth signaling and replicative potential to impaired energy metabolism is in recognizing that proliferation, rather than quiescence, is the default state of both microorganisms and metazoans (3, 6–9). The default state of the cell is the condition under which cells are found when they are freed from any active control. Respiring cells in mature organ systems are largely quiescent because their replicative potential is under negative control through the action of normal mitochondrial function. Respiration maintains differentiation and quiescence. In addition, tumor suppressor genes such as *p*53 and the retinoblastoma protein, pRB, can also help to maintain quiescence (6, 10). As *p*53 function is linked to cellular respiration, prolonged respiratory insufficiency will gradually reduce *p*53 function, thus inactivating the negative control of *p*53 and other tumor suppressor genes on cell proliferation. In contrast to the view of Hanahan and Weinberg that quiescence is the default state of cells, I believe that proliferation is the default state of cells. Sonnenschein and Soto (7) provide an excellent review describing how proliferation is the default state of metazoan cells.

A persistent impairment in respiratory function will trigger the RTG response, which is necessary for upregulating fermentation pathways needed to maintain the $\Delta G'_{ATP}$ for viability. The RTG response will activate numerous oncogenes such as *MYC*, *Ras*, *HIF-1α*, *Akt*, and *m-Tor*, which are required to sustain fermentation as the prime energy source in the presence of insufficient respiration (11–15). Glucose and glutamine become the main energy substrates for driving fermentation through glycolysis and TCA cycle substrate-level phosphorylation. Salvadore Moncada and colleagues showed how glucose and glutamine are linked to the cell cycle and proliferation (16). This is important, as glucose and glutamine are the main energy metabolites needed for fermentation and tumor growth.

In addition to facilitating the metabolism of glucose and glutamine through fermentation and substrate-level phosphorylation, MYC and Ras also stimulate cell proliferation (17–19). Part of this mechanism also includes inactivation of pRB, the function of which is dependent on mitochondrial activities and the cellular redox state (10). Disruption of the pRB signaling pathway will contribute to cell proliferation and neoplasia (2). Cell proliferation is linked to fermentation, whereas quiescence is linked to respiration (9, 20). Unlike normal cells, which engage respiration following proliferation, tumor cells remain dependent on glucose and glutamine for fermentation because their OxPhos is insufficient to maintain homeostasis. Hence, the growth signaling abnormalities and limitless replicative potential of tumor cells can be linked directly to the requirements of fermentation energy, which originates ultimately from impaired or insufficient respiration.

It is also interesting that the RTG response underlies replicative life span extension in budding yeast. Yeast longevity is linked to the number of buds that a mother

cell produces before it dies (14). The greater the loss of mitochondrial function, the greater the induction of the RTG response and the greater the longevity (bud production) in yeast (21). As mitochondrial energy efficiency declines with age, fermentation energy becomes necessary to compensate for insufficient respiration. A reliance on fermentation becomes essential if a cell is to remain alive. A greater dependency on substrate-level phosphorylation will induce oncogene expression and unbridled proliferation, which could underlie in part the enhanced longevity in yeast (14, 22, 23). When this process occurs in mammalian cells, however, the phenomenon is referred to as *neoplasia* or *new growth*. We proposed that replicative life span extension in yeast and the limitless replicative potential in tumor cells can be linked through common bioenergetic mechanisms involving impaired mitochondrial function (3). It is insufficient respiration that underlies yeast longevity as well as the origin of cancer.

LINKING TELOMERASE ACTIVITY TO CELLULAR ENERGY AND CANCER

Telomerase is a ribonucleoprotein enzyme complex associated with cellular immortality through telomere maintenance. Telomerase is activated in about 90% of human cancers, suggesting a role in tumorigenesis (24–26). Emerging evidence suggests that mitochondrial dysfunction could underlie the relocation of telomerase from the mitochondria, where it seems to have a protective role, to the nucleus, where it maintains telomere integrity necessary for limitless replicative potential (27–29). Interestingly, telomerase activity is high during early embryonic development when cell proliferation is high, but telomerase activity is low in adult tissues where most cells are differentiated and quiescent (30, 31).

These findings suggest a linkage of telomerase activity with energy metabolism. Telomerase activity is high in normal cells or tumor cells that primarily use fermentation for energy, but the activity is low or nonexistent in nontumorigenic differentiated cells that primarily use OxPhos for energy. These findings suggest that the energy state of the cells dictates the activity level of telomerase. Elevated telomerase activity in tumor cells would therefore be an effect rather than a cause of cancer. Further studies will be necessary to determine how changes in telomerase expression and subcellular localization could be related to mitochondrial dysfunction, elevated fermentation, and the limitless replication of tumor cells.

EVASION OF PROGRAMMED CELL DEATH (APOPTOSIS)

Apoptosis is a coordinated process that initiates cell death following a variety of cellular insults. Damage to mitochondrial energy production is one type of insult that can trigger the apoptotic cascade, which ultimately involves release of mitochondrial cytochrome *c*, activation of intracellular caspases, and death (2, 3). In contrast to normal cells, acquired resistance to apoptosis is a hallmark of most types

of cancer cells (2). The evasion of apoptosis is a predictable physiological response for tumor cells that primarily use fermentation and substrate-level phosphorylation for energy production following respiratory damage during the protracted process of carcinogenesis (32). Only those cells capable of making the gradual energy transition from respiration to fermentation in response to respiratory insufficiency will be able to evade apoptosis. Cells unable to make this energy transition will die and thus never become tumor cells. The ability of a cell to replace respiration with fermentation is a central tenet of the Warburg theory (20, 33–35).

Numerous findings indicate that the genes and signaling pathways needed to upregulate and sustain fermentation are themselves antiapoptotic. For example, sustained glycolysis or glutamine fermentation requires participation of mTOR, MYC, Ras, HIF-1a, and the IGF-1/PI3K/Akt signaling pathways (13–15, 22, 32, 36, 37). The upregulation of these genes and pathways together with inactivation of tumor suppressor genes such as *p*53, which is required to initiate apoptosis, will disengage the apoptotic signaling cascade thus preventing programmed cell death (3, 38).

Abnormalities in the outer mitochondrial membrane and inner membrane potential ($\Delta\Psi_m$) can also induce expression of known antiapoptotic genes (*Bcl2* and *Ccl-X*$_L$) (32, 39). Tumor cells will continue to evade apoptosis as long as they have access to glucose and glutamine, which are required to maintain fermentation energy. Glycolytic tumor cells, however, can readily express a robust apoptotic phenotype if their glucose supply is targeted. We clearly showed that dietary or calorie restriction could significantly increase the number of apoptotic cells in experimental brain tumors (15, 40, 41). This is also thought to contribute in part to the therapeutic action of dietary energy reduction in managing human glioblastoma (42–44). Hence, the evasion of apoptosis in tumor cells is linked directly to a dependency on fermentation and substrate-level phosphorylation for energy, which is itself a consequence of impaired respiratory function.

SUSTAINED VASCULARITY (ANGIOGENESIS)

Angiogenesis involves neovascularization or the formation of new capillaries from existing blood vessels and is associated with the processes of tissue inflammation, wound healing, and tumorigenesis (45–48). Figure 1.3 highlights the role of angiogenesis in tumor progression. Angiogenesis is required for most tumors to grow beyond an approximate size of 0.2–2.0 mm (49). This is necessary to provide the tumor with essential energy nutrients, including glucose and glutamine, and to remove toxic tumor waste products such as lactic acid and ammonia (3, 50).

In addition to its role in upregulating glycolysis in response to hypoxia, HIF-1α is also the main transcription factor for vascular endothelial growth factor (VEGF), which stimulates angiogenesis (15, 51–53) (see also Figs. 17.11 and 17.16). HIF-1α is part of the IGF-1/PI3K/Akt signaling pathway that also indirectly influences the expression of βfibroblast growth factor (FGF), another key angiogenesis growth factor (15, 54). Many of the genes and metabolites that drive angiogenesis arise

as secondary consequences of tumor cell fermentation. Hence, the sustained vascularity of tumors can be linked mechanistically to the metabolic requirements of fermentation and substrate-level phosphorylation necessary for tumor cell survival.

When viewed collectively, the information presented in this chapter provides compelling evidence linking several of the Hanahan and Weinberg hallmarks of cancer directly to insufficient respiration in tumor cells. It is also interesting that the cancer hallmarks discussed in this chapter are not unique to malignant cancers but are also present in benign tumors (55). Indeed, abnormalities in growth control, telomerase activity, apoptosis, and angiogenesis are present in many tumors that do not invade or metastasize. Nevertheless, I think it is easy to see how respiratory insufficiency can account for these characteristics in tumors.

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