Review Article | DOI 10.3349/ymj.2010.51.5.617 pISSN: 0513-5796, eISSN: 1976-2437 | Yonsei Med J 51(5):617-621, 2010 | YMJ

Perspective beyond Cancer Genomics: Bioenergetics of Cancer Stem Cells

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Received: January 5, 2010
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· The authors have no financial conflicts of interest.

Although the notion that cancer is a disease caused by genetic and epigenetic alterations is now widely accepted, perhaps more emphasis has been given to the fact that cancer is a genetic disease. It should be noted that in the post-genome sequencing project period of the 21st century, the underlined phenomenon nevertheless could not be discarded towards the complete control of cancer disaster as the whole strategy, and in depth investigation of the factors associated with tumorigenesis is required for achieving it. Otto Warburg has won a Nobel Prize in 1931 for the discovery of tumor bioenergetics, which is now commonly used as the basis of positron emission tomography (PET), a highly sensitive noninvasive technique used in cancer diagnosis. Furthermore, the importance of the cancer stem cell (CSC) hypothesis in therapy-related resistance and metastasis has been recognized during the past 2 decades. Accumulating evidence suggests that tumor bioenergetics plays a critical role in CSC regulation; this finding has opened up a new era of cancer medicine, which goes beyond cancer genomics.

Key Words: Cancer, genetics, bioenergetics, cancer stem cells

INTRODUCTION

According to the modern understanding of cancer, it is a disease that is primarily associated with genetic and epigenetic alterations.[1] Numerous studies, including our earlier works, have supported the notion that carcinogenesis involves the activation of tumor-promoting oncogenes and the inactivation of growth-inhibiting tumor suppressor genes. However, extensive research is warranted in two areas, namely, tumor bioenergetics and the cancer stem cell (CSC) hypothesis, which did not receive the required attention after the success of the genome sequencing project ofthe 21st century. An investigation of these two concepts would give rise to a new era in the study of cancer biology. Indeed, recent studies have indicated that the two apparently distinct fields might be related to each other and can converge more rapidly than previously recognized.

TUMOR BIOENERGETICS

The Warburg effect

Otto Warburg won a Nobel Prize in 1931 for his work on respiratory impairment in cancer. Warburg showed that unlike normal tissues that derive most of their

ATP by metabolizing glucose to carbon dioxide and water, which is an oxygen-dependent process performed by the mitochondria, cancer cells rarely depend on mitochondria for respiration and obtain almost half of their ATP by directly metabolizing glucose to lactic acid, even in the presence of oxygen.[2] However, with the discovery that tumors do not show any shift to glycolysis,[3] Warburg's cancer theory (high lactate production and low mitochondrial respiration in tumor under normal oxygen pressure) was gradually discredited. The ascendancy of molecular biology over the last quarter of the century has placed more emphasis on the genetic alterations of cancer cells, and eclipsed the study of tumor bioenergetics, including Warburg's ideas.

Significance of the Warburg effect

The increasing number of recent reports on the Warburg effect has reestablished the significance of this effect in tumorigenesis, indicating that bioenergetics may play a critical role in malignant transformation. Furthermore, it has been reported that *TP53*, which is one of the most commonly mutated genes in cancer, can trigger the Warburg effect.[4] Glycolytic conversion is initiated in the early stages in cells that are genetically engineered to become cancerous, and the conversion was enhanced as the cells became more malignant.[5] Therefore, the Warburg effect might directly contribute to the initiation of cancer formation not only by enhanced glycolysis but also via decreased respiration in the presence of oxygen, which suppresses apoptosis.[6] This effect may also produce a metabolic shift to enhanced glycolysis and play a role in the early stages of multistep tumorigenesis *in vivo*.[7]

Embryonic stem (ES) cells and immortalized primary and cancerous cells show the common concerted metabolic shift, including enhanced glycolysis, decreased apoptosis, and reduced mitochondrial respiration; however, the mechanism underlying this shift is poorly characterized.[7] This finding reinforces the use of somatic stem cells or metastatic tumor cells in hypoxic niches. Hypoxia appears to regulate the functions of hematopoietic stem cells in the bone marrow[8] and metastatic tumor cells (M. Mori, unpublished data) by preserving important stem cell functions, such as cell cycle control, survival, metabolism, and protection against oxidative stress.

However, this idea is still a controversial topic;[3] one of the arguments suggest that the Warburg effect is the consequence of cancer, and not the main contributing factor of the disease. Nevertheless, several companies and laboratories, including ours, are now attempting to evaluate the bioenergetics associated with tumorigenesis by testing and challenging the available anticancer drugs.

The Warburg effect is now the basis for positron emission tomography (PET), a highly sensitive noninvasive technique used in pre-clinical and clinical imaging of cancer biology; this technique has facilitated early diagnosis and better management of oncology patients.[9] With greater acceptance, it should become an increasingly important technique for cancer imaging in the next decade.[9]

CANCER STEM CELL HYPOTHESIS

The hypothesis

In 1937, Furth and Kahn[10] showed that leukemia can be initiated in mice using a single tumorigenic cell. This gave rise to a notion that a single or a few malignant cells, which have been transformed from normal somatic cells, can produce tumors. During the turn of the 21st century, the CSC hypothesis has gained recognition again, mainly in the Western world. After the identification of rare CSCs in leukemia,[11-13] molecular markers for detecting CSCs in solid tumors, such as head and neck,[14] breast,[15] and brain cancers,[16,17] have also been identified. The research team at one of our laboratories has obtained the first evidence of CSCs in the gastrointestinal system,[18] and our findings have subsequently been confirmed by other researchers.[19,20]

Significance of the hypothesis

A small population of cancer-initiating cells plays a very important role, in that it may cause resistance to chemotherapy or radiation therapy or lead to post-therapy recurrence even when most of the cancer cells appear to be dead.[21] In addition to their genetic alterations, CSCs are believed to mimic normal adult stem cells with regard to properties like self renewal and undifferentiated status, which eventually leads to the formation of differentiated cells.[22] Moreover, unlike well-differentiated daughter cells, small populations of CSCs are believed to be more resistant to toxic injuries and chemoradiotherapy.[23] Whereas the conventional cancer therapies have always been targeted toward proliferating cells, the control of CSCs, which is often exercised in the dormant phase of the cell cycle, can now be applied to achieve complete tumor regression.

Identification of cancer-specific markers

Due to their potential use in clinical applications, the surface markers of CSCs have been studied and identified. Adult stem cells and their malignant counterparts share similar intrinsic and extrinsic factors that regulate the self renewal, differentiation, and proliferation pathways.[24] The following are the examples of candidate markers: musashi-1 (Msi-1),[25] hairy and enhancer of split homolog-1 (Hes-1),[26] CD133 (prominin-1, Prom1),[27,28] epithelial cellular adhesion molecule (EpCam),[29] claudin-7,[29] CD44 variant isoforms,[29] Lgr5,[30]

Hedgehog (Hh),[31] bone morphogenic protein (Bmp),[32,33] Notch,[34] and Wnt.[35] Nevertheless, little is known about the molecular markers that are characteristic of dormant stem cells and amplified populations of differentiating cells of solid tumors, such as the tumors of the gastrointestinal tract.[35]

BIOENERGETICS OF CANCER STEM CELLS

The bioenergetics associated with the adaptation of CSCs to their microenvironment still requires extensive research. Although numerous studied suggested the association between Warburg effect and reduced oxidative stress in cancer, the relevant molecular mechanism was not known until very recently when Ruckenstuhl, et al.[6] reported their findings in a yeast model.

Hypoxic adaptations in the presence of oxygen

Through different biochemical and biophysical pathways, which are characteristic to cancer cells, tumor cells adopt this phenotype, i.e., high glycolysis and decreased respiration, in the presence of oxygen. It has been shown that although the induction of hypoxia and cellular proliferation engage entirely different cellular pathways, they often coexist during tumor growth.[36] The ability of cells to grow during hypoxia results, in part, from the crosstalk between hypoxia-inducible factors (Hifs) and the proto-oncogene c-Myc.[36] These genes partially regulate the development of complex adaptations of tumor cells growing in low O_2, and contribute to fine tuning the adaptive responses of cells to hypoxic environments.[36] Nevertheless, how cancer cells achieve one of the most common phenotypes, namely, the "Warburg effect," i.e., elevated glycolysis in the presence of oxygen, is still a topic of hypothesis, unless the involvement of glycolysis genes is considered.

Recently, it was shown that the hexokinase 2 (Hk-2) protein, its mitochondrial receptor, namely, voltage-dependent anion channel (Vdac), and the gene encoding Hk-2 play the most pivotal and direct roles in the "Warburg effect," despite some impairment in the respiratory capacity of malignant tumors is involved.[37] Furthermore, metabolic reprogramming during physiologic cell proliferation and tumorigenesis may alter cell growth and proliferation by modifying the flux of cellular mediators of signal transduction and gene expression, including the expression of phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR system, hypoxia-inducible factor 1 (Hif-1), and Myc.[38] In particular, the genes of many glycolysis enzymes are under the control of Myc, Hif-1, and tumor suppressor p53,[7] suggesting that enhanced glycolysis is essential for both immortalization and transformation, since it renders cells resistant to oxidative stress and adaptive to hypoxic condition.[7]

Low oxidant levels in the niche

A study on hematopoietic stem cells revealed that low levels of reactive oxygen species are present in the bone marrow.[39] A low-oxygen niche in the bone marrow limits reactive oxygen species production, thus providing hematopoietic stem cells with a long-term protection from reactive oxygen species stressors such as senescence, apoptosis, and DNA damage.[39] The research indicated that it is possible to isolate the early hematopoietic stem cell population by taking advantage of limited intracellular reactive oxygen species activity.[39] Thus, somatic stem cells such as those in the hematopoietic system reside in the hypoxic area in the bone marrow niche, which affords them protection from deleterious damages, presumably through the involvement of glycolytic metabolism.[39,40]

The Warburg effect has been observed in differentiating cancer cells (e.g., cells that undergo epithelial-to-mesenchymal and mesenchymal-to-amoeboid transition), cells resistant to anoikis, and cells which interact with the stromal components of the metastatic niche.[41] We showed that the epithelial-to-mesenchymal transition is involved in the resistance to chemotherapy in gastrointestinal cancer cells.[42] Cancer metastasis can be regarded as an integrated "escape program" triggered by redox changes.[41] These alterations might be associated with avoiding oxidative stress in the niche of the tumor cells, or presumably with the response to treatments aimed at genetic targets, such as chemotherapy and radiation. Regulation of reactive oxygen species in CSCs population is an important issue; we are investigating this topic by *in vitro* and *in vivo* experiments.

CONCLUSION

We studied the significance of bioenergetics of CSCs. Although the accomplishments of the genome project have contributed to cancer research and medicine, we have to pay more attention inimproving cancer diagnosis and therapy. In this article, we have highlighted the significance of a few relevant concepts, which have been recently discovered. Moreover, our study indicated that the introduction of induced pluripotent stem (iPS) cell genes was necessary for inducing the expression of immature status-related proteins in gastrointestinal cancer cells, and that the induced pluripotent cancer (iPC) cells were distinct from natural cancer cells with regard to their sensitivity to differentiation-inducing treatment.[43] For the complete eradication of cancer, however, future efforts should be directed toward improving translational research.

ACKNOWLEDGEMENTS

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; and from Mitsubishi Pharma Research Foundation, Uehara Memorial Foundation, and Kobayashi Cancer Foundation, Japan.

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