Hypoxic markers in non-small cell lung cancer (NSCLC) – A review

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Abstract. – Surgery, chemotherapy and radiotherapy have been the main pillars of cancer treatment. Some of the recent improvements in survival of other cancers can be attributed to novel treatment therapies. Such therapies mostly target specific molecules involved in cancer progression and metastasis. Development and clinical introduction of targeted therapies involve identification of new and potentially important molecules in cancer progression. The next important step is to evaluate its prognostic value. Prognostication by molecular markers is also important as this may identify subgroups of patients in need for additional treatment or not, which was not possible with the traditional clinic-pathological prognosticators. Hypoxic markers have been widely explored in the recent past for their prognostic efficacy in non-small cell lung cancer.

The present review article will enlighten importance of hypoxic markers with special reference to non-small cell lung carcinoma.

Key Words:
  Hypoxia, Markers, Lung cancer, NSCLC.

Introduction

Lung cancer is by far the prominent cause of mortality due to cancers in the western world. As far as causes are concerned, cigarette smoking is by far the most important etiologic factor responsible for the same. Due to the high content of carcinogens in cigarette it is by far the largest voluntarily source of human exposure to carcinogens (1). Other known carcinogens for lung cancer include asbestos, radon, arsenic, cadmium and chromates[2]. Never smokers account for up to 25% of lung cancer patients and has recently been suggested as a separate entity due to differences in epidemiological, clinical and molecular characteristics[3].

There are currently no generally implemented screening programs for lung cancer. Recently, the first results from the randomized NCI-sponsored National Lung Screening Trial (NLST) were published in NEJM[4]. US citizens between 55-74 years of age with a history of heavy smoking were randomly assigned to a yearly low-dose CT or regular chest radiography. Although the results are premature and there is a concern of over-diagnosis, there was significantly reduced lung cancer mortality in the CT screened population. So, screening plays an important role in effective management of cancer. The present review article will focus on the two important upcoming prognostic factors viz. hypoxia and angiogenesis. These factors are being explored worldwide for their prognostic importance in lung cancer.

Hypoxia in Cancer

Oxygen is a vital part of human metabolism as the presence of oxygen enables the cells to retrieve around 16 times more energy out of glucose than what is otherwise possible under anaerobic conditions. In growing tissue during development or as in tumors, there is continuously changing oxygen pressure and these cells have to adapt themselves for these conditions to survive and proliferate. In lung tumors, median tumor oxygen tension has been measured at 2.2% (range 0.1-6%), indicating hypoxia to be a prevalent feature[5]. Based on histological studies of lung tumors, it was proposed in the earlier studies that the necrosis, found in cores surrounded by viable cells and a capillary vessel, was the result of hypoxia[6]. Later, hypoxia became important parameter to be measured more precisely by electrodes. However, due to considerable inter- and intra-tumoral variability, there was always a priority demand for more precise and dynamic scoring of hypoxia[7,8]. Additionally, this is probably even more complicated as hypoxia is not only a chronic feature, but can also be acute due to changing dynamics of blood flow. Furthermore, cancer cells with one type of hypoxia may have a

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different treatment response than cells with the other type[9,10]. Hypoxia is a cardinal phenotype of the tumor environment, both due to tumor respiration and due to neoplastic cell colonization of tissues without a prerequisite blood supply.

The low oxygen tension triggers the gene expression towards a more aggressive phenotype and hypoxia reduces the sensitivity to therapy. These characteristics are the result of activation of key hallmarks of cancer like angiogenesis, metastasis, increased DNA replication and proliferation in both hypoxia induced factor (HIF). The observation by Schwarz et al[11] stated that hypoxic cells are less sensitive to radiotherapy, is more than 100 years old. Almost 50 years later, Gray et al[12] managed to establish evidence for the radioresistance seen in hypoxia *in vitro*. Moreover, another study[13] found decreased tumor cell survival in irradiated lymphosarcomas from mice breathing hyperbaric O_2 and similar results were observed by Churchill-Davidson[14] in the cancer patients. Although hypoxia is recognized as a key feature of resistance to radiotherapy, the later hypoxia-modifying studies with hyperbaric O_2 and hypoxic cell radiosensitizers, have in general been disappointing, thus hypoxic modification is normally not influencing clinical practice[15]. Hypoxia is also known to mediate resistance to chemotherapy, both directly and indirectly through the raised interstitial fluid pressure (IFP)[16,17].

Hypoxia Related Biomarkers

Hypoxia induced factor (HIF) is the active heterodimer of HIF α and HIF β. HIF α is one of the proteins with the shortest known half-life, but also detectable in less than 2 minutes after exposure to hypoxia[18]. Moreover, HIF α was the first HIF family member to be described and the most widely studied. It is ubiquitously expressed and induces a wide range of hypoxia-inducible genes. It is highly expressed in many different tumors, but infrequent in most normal tissues[19,20]. Over expression of HIF α has consistently been found associated with a poor prognosis in a broad range of tumors including NSCLC[21,22]. Also the HIF β isoform seems important in physiology as targeted disruption of HIF β leads to embryonic lethality. Although, both HIF α and HIF β share a significant sequence homology but they differ in tissue distributions and effects on tumor progression. Whereas HIF α is found almost in every tissue, HIF β expression is more restricted and seems to be highly expressed in tissues mainly involved in systemic delivery of O_2, like lung, heart and endothelium. Regarding target genes, HIF α uniquely stimulates the expression of many enzymes like lactate dehydrogenase 5 (LDH5) and carbonic anhydrase IX (CAIX), while transforming growth factor and erythropoietin (EPO) are up-regulated in hypoxia by HIF β. Other transcriptional genes like glucose transporter 1 (*GLUT1*) and *VEGF-A* are commonly upregulated by both subunits[23].

GLUT1 in Lung Cancer

Sugars are an important substrate for energy production through cellular respiration with oxidative phosphorylation as the final and most productive step. However, cancer cells mainly exploit glycolysis as discovered by Warburg more than 50 years ago[24]. In fact, cancer cells actually prefer glycolysis with or without the presence of oxygen. To achieve this phenotype, an increased import of sugars, mainly glucose, is needed. Other sugars like fructose can be used, but glucose uptake is the rate-limiting step. Sugars are hydrophilic and need to be transported into cells by glucose transporters. Transportation is mediated through membranes by membrane proteins such as facilitated glucose transporters (GLUTs) or sodium/glucose cotransporters (SGLT). There are several subtypes of these sugar transporters, but GLUT1 is responsible for the basal glucose uptake which is probably why it is the most studied glucose transporter in cancer. It is also related to the rate of glucose metabolism and is expressed in all tissues. GLUT1 is induced by hypoxia[25], but it is also known to be regulated by c-Myc[26].

CAIX in Cancer

Cellular biochemistry can be significantly altered by small changes in pH. Furthermore, proper regulation is vital for survival and function of cells. Cancer cells are characterized by a high metabolism and therefore must be able to handle the high intracellular production of excess protons (H^+) by transporting them from the inside of the cells to the extracellular environment. This can be achieved by Na^+/H^+-exchange, H^+-lactate co-transport or HCO_3 (bicarbonate) dependent buffering with a subsequent extracellular CO_2 diffusion. For the bicarbonate-dependent buffering to be efficient, catalysis by carbonic anhydrases is imperative. In cancer, the extracellular bound carbonic anhydrase IX (CAIX) has been of increasing interest due to its induction by hypoxia and its expression has been found in many

cancers. Expression of CAIX is related to hypoxia below 1% O_2 and is therefore seen expressed between 80-130 □m from blood vessels[27]. Among the molecules related to hypoxia, CAIX has been proposed as one of the most reliable markers of hypoxia although this is still a matter of controversy.

LDH5 in Lung Cancer

Due to the high metabolic rate of cancer cells by glycolysis and the use of citric acid (TCA) intermediates for cancer cell membrane composition, there is a high production of pyruvate not needed for further energy production. Hence, pyruvate is converted to lactate by lactate dehydrogenases. Among five isoenzymes, lactate dehydrogenase 5 (LDH5), also called LDHA, has the highest efficiency in catalyzing pyruvate to lactate. Lactate can subsequently be transported to the extracellular space by a monocarboxylate transporter. LDH5 is also induced by HIF1alpha and is overexpressed in common cancers like NSCLC(67;68) head and neck cancers, non-Hodgkin B-cell lymphomas and colorectal cancers[28-30].

Angiogenesis in Cancer

Angiogenesis is the physiological phenomenon of growth of new vessels from pre-existing vessels as opposed to vasculogenesis where blood vessels are formed without pre-existing ones[31]. Oxygen and nutrients are brought to the tissues by diffusion and blood supply. Folkman[32] stated in 1971 that angiogenesis was crucial if tumors were to grow beyond 1-2 mm^3 and proposed the idea of targeting angiogenesis. Angiogenesis is a complex and dynamic process tightly regulated by growth factor families like vascular endothelial growth factor (VEGF), placental growth factor (PLGF) plasma hepatocyte growth factor, platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and their receptors[33]. Moreover, players in other pathways like the angiopoietins (Ang) and their Tie-2 receptor, NOTCH-Deltalike ligand 4 (DLL4) and endogenous angiogenesis inhibitors like vasohibin, angiostatin, endostatin, trombospondin-1 and tumstatin[34] are also associated with angiogenesis.

Conclusions

So, from the above discussion it is clear that hypoxic markers are crucial in lung cancer. Further studies are required in the area to affirm their use as gold standard biomarkers for efficient as well as early diagnosis of lung cancer. Moreover, similar studies can be excuted for other cancer types too, so as to make hypoxic markers as universal marker for cancer diagnosis in clinical setting.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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