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Research Paper

Positron emission tomography-computed tomography and 4-hydroxynonenal-histidine immunohistochemistry reveal differential onset of lipid peroxidation in primary lung cancer and in pulmonary metastasis of remote malignancies

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ABSTRACT

The Aim of the study was to reveal if PET-CT analysis of primary and of secondary lung cancer could be related to the onset of lipid peroxidation in cancer and in surrounding non-malignant lung tissue.

Methods: Nineteen patients with primary lung cancer and seventeen patients with pulmonary metastasis were involved in the study. Their lungs were analyzed by PET-CT scanning before radical surgical removal of the cancer. Specific immunohistochemistry for the major bioactive marker of lipid peroxidation, 4-hydroxynonenal (HNE), was done for the malignant and surrounding non-malignant lung tissue using genuine monoclonal antibody specific for the HNE-histidine adducts.

Results: Both the intensity of the PET-CT analysis and the HNE-immunohistochemistry were in correlation with the size of the tumors analyzed, while primary lung carcinomas were larger than the metastatic tumors. The intensity of the HNE-immunohistochemistry in the surrounding lung tissue was more pronounced in the metastatic than in the primary tumors, but it was negatively correlated with the cancer volume determined by PET-CT. The appearance of HNE was more pronounced in non-malignant surrounding tissue than in cancer or stromal cells, both in case of primary and metastatic tumors.

Conclusions: Both PET-CT and HNE-immunohistochemistry reflect the size of the malignant tissue. However, lipid peroxidation of non-malignant lung tissue in the vicinity of cancer is more pronounced in metastatic than in primary malignancies and might represent the mechanism of defense against cancer, as was recently revealed also in case of human liver cancer.

1. Introduction

The basic feature of a tumor cell is the metabolic switch from oxidative phosphorylation to anaerobic glycolysis even under circumstances of normal oxygen saturation, a phenomenon known as the Warburg effect [1]. Lactate dehydrogenase (LDHA) catalyzes the conversion of pyruvate to lactate, and is considered the key enzyme that regulates anaerobic metabolism, including the increased expression of the Glut-1 to Glut-4 transport proteins [2,3]. The mentioned tumor cell feature is used in the diagnostic method of Positron Emission Tomography and Computed Tomography (PET-CT) scanning. Instead of glucose, the glucose analog 18-fluorodeoxyglucose

Abbreviations: PET-CT, Positron Emission Tomography-Computed Tomography; 4-HNE, 4-hydroxynonenal; 18F-FDG, 18-fluorodeoxyglucose; SPN, solitary pulmonary nodule; LDHA, Lactate dehydrogenase A; SUV, standardized uptake value; ROS, reactive oxygen species; RNS, reactive nitrogen species; RALBP1, Ral-binding protein1; Nrf2, nuclear factor erythroid 2-related factor; Keap1, Kelch-like ECH-associated protein 1; TAM, tumor-associated macrophages; VEGF, vascular endothelial growth factor; NSCLC, non-small cell lung cancer; VATS, video-assisted thoracoscopic surgery; GSH, glutathione; mAb, monoclonal antibodies; hydroxyl radical $OH^{\cdot}$, superoxide $O_2^{\cdot-}$; N, nitrogen oxide; NO_2, nitrogen dioxide; H_2O_2, hydrogen peroxide; HOCl, hypochloric acid; O_3, ozone; NADPH, nicotinamide adenine dinucleotide phosphate Nox oxidase; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinases

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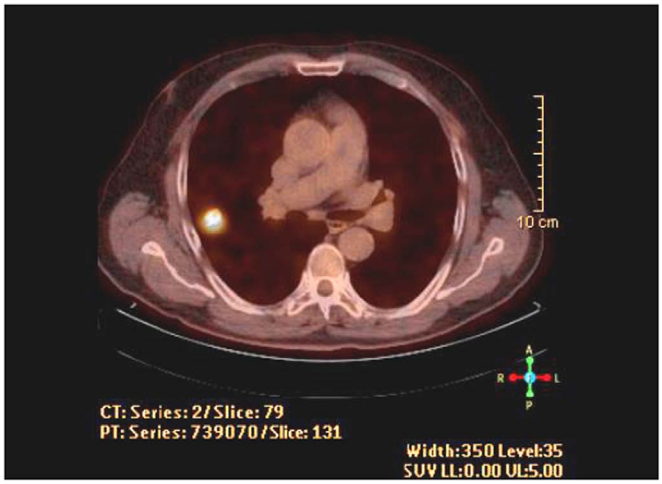

Fig. 1. PET-CT scan of solitary malignant pulmonary nodule reflecting cancer growth as shining spot (SUVmax 5.0).

(18F-FDG) is used. Cancer cells phosphorylate 18F-FDG by hexokinase to FDG-6-phosphate, a compound which is not further metabolized but accumulates in the cell. The intensity of 18F-FDG accumulation is quantitatively expressed by standardized uptake value (SUVmax) [4–6]. The metabolic activity of the tumor expressed as SUVmax is in correlation with the intensity of anaerobic glycolysis of malignant cells and the number of metabolically active cancer cells. In the majority of clinical studies, a SUV of >2.5 is considered the cut-off value indicating a malignant etiology of the pulmonary nodule [7–12] (Fig. 1).

Oxidative stress is defined as an imbalance in the cellular redox reactivity towards oxidation, resulting in an increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen and nitrogen species include various substances like the oxygen free radicals superoxide (O_2^-) and hydroxyl radical (OH^-), the nitrogen free radicals nitrogen oxide (NO) and nitrogen dioxide (NO_2), as well as molecules such as hydrogen peroxide (H_2O_2), hypochloric acid (HOCl) and ozone (O_3). Among ROS the most reactive are free radicals, which contain an unpaired electron in their outer shells [13,14]. ROS may lead to irreversible damage in macromolecules such as DNA, proteins and lipids, where the occurrence of self-catalyzed chain reaction of lipid peroxidation plays a particular role, with the subsequent production of reactive aldehydes out of which 4-hydroxynonenal (HNE) is of major interest due to its numerous biological effects. Thus, HNE is known also as “second messenger of free radicals", as it lives longer than free radicals and can migrate within the cell gaining biological activities that resemble free radicals. Additionally, HNE is able to bind to macromolecules and thereby modify their function even if not being toxic [15,16]. The effects of HNE are ranging from cell growth regulation, intracellular signal transmission and communication with the cellular environment eventually leading to cytotoxicity and cell death, which mainly depend on concentrations of HNE, the character of the biomolecules involved and the type of cells affected by HNE [17–21]. In various malignant cells ROS levels are permanently elevated due to the malignant cell transformation and increased metabolism, whereas their antioxidant levels are often decreased [22–24]. Although such unfavorable conditions should normally lead to apoptosis and cell death, instead uncontrolled tumor cell proliferation occurs, while the tumor-specific antioxidant protection system neutralizes the high ROS levels. A possible cause could be an alternative antioxidant protection route by the multispecific transport protein Ral-binding protein1 (RALBP1) and its conjugation potential with the HNE molecule [25], as well as the increased activity of transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) responsible for antioxidant synthesis within the cell. Increased Nrf2 activity has been noted in malignant lung tumor cells, and is in correlation with chemoresistance and radioresistance of tumors. Increased Nrf2 activity in malignant cells is the result of a mutation in the cytoplasmic molecule Keap1 (Kelch-like ECH-associated protein 1), which in conjunction with Nrf2 causes its inactivation [26–28]. Hence, the multipotent role of the HNE molecule is demonstrated by its potential to create a HNE-Keap1 complex [29], as well as the HNE-His type of protein adducts, thereby directly affecting the tumor antioxidant protection system, but also causing inflammation [30].

The association between chronic inflammation and tumors has been proven repeatedly in numerous experimental and epidemiological studies. An important feature of tumors is their potential for chemotaxis and inflammatory cell activation [31]. The role of tumor-associated macrophages (TAM) has been thoroughly researched. The activated macrophages show an increased expression of the enzyme group nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) and inducible nitric oxide synthase (iNOS). They stimulate tumor invasion by releasing the matrix metalloproteinases MMP-2 and MMP-9, which break down the extracellular matrix and basal membrane [32]. TAMs also stimulate tumor angiogenesis by releasing vascular endothelial growth factor (VEGF) and endothelin-2 urokinase-type plasminogen activator [33,34]. The host-to-tumor relationship can therefore be manifested by increased accumulation of inflammatory cells in the vicinity of the tumor and the release of ROS and second messengers of oxidative stress, such as HNE molecules, which have the potential to affect the signaling pathways in cancer cells [35,36].

By comparing the metabolic activity of the tumor in different PET/CT scans with the immunohistochemistry findings of HNE in the malignant cells, tumor stroma and in normal tissue adjacent to the tumor, the aim of this research was to determine whether the tumor affects the surrounding tissue by the spread of oxidative stress or whether the surrounding tissue aims to affect the tumor as a reflection of the host reaction, by way of an inflammatory reaction and HNE generated within malignant tissue and in the surrounding non-malignant tissue.

2. Patients, materials and methods

2.1. Patients

Nineteen patients (52.8%) with primary lung tumors and 17 patients (47.2%) with secondary lung tumors were involved in this study.

In the group of patients with primary lung tumors majority suffered from adenocarcinoma, followed by planocellular carcinoma, non-small cell lung cancer (NSCLC), and carcinoid, while one patient was diagnosed with mesothelioma. Two out of three patients were current or former smokers, while every third patient received platinum-based chemotherapy prior to lung resection (Table 1).

In the group with secondary lung tumors, 13 patients had been pathohistologically diagnosed with metastatic adenocarcinoma of the rectosigmoid colon. The other diagnoses included metastatic breast, kidney, and laryngeal cancer. Every third patient was current or former smoker, while most of them had undergone chemotherapy prior to lung resection. The mean time from the last chemotherapy cycle to resection was 18.8 months (Table 2).

The tissue samples for pathohistological analysis were obtained after lung lobectomy or segmentectomy performed at the Department of Thoracic Surgery of the Clinical Hospital Dubrava in Zagreb. The most frequently used surgical technique was video-assisted thoracoscopic surgery (VATS), and in several patients thoracotomy was necessary due to the extent of the procedure. The indication for open biopsy of the tumor growth was established by the thoracic surgeon and the attending pulmonologist. The basic requirement for enrollment into this prospective study were positive PET-CT scans of pulmonary