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Metals distribution in colorectal biopsies: New insight on the elemental fingerprint of tumour tissue

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ABSTRACT

Background: Some studies have linked colorectal cancer to metal exposure.
Aims: Our objective was to evaluate the element distribution in colorectal adenocarcinoma biopsies, adjacent non-tumour tissues, and healthy controls.
Methods: The study is a case–control study which compared the element distribution in colon biopsies from two groups of patients: with colorectal cancer (2 types of samples: colorectal cancer biopsies and adjacent non-tumour tissues) and healthy controls. Fifteen metal concentrations (Aluminium, Boron, Cadmium, Chromium, Copper, Iron, Magnesium, Manganese, Nickel, Lead, Selenium, Silicon, Titanium, Vanadium, and Zinc) were quantified by using inductively coupled plasma atomic emission spectrometry.
Results: 104 patients were included: 76 in the colorectal cancer group, 28 in the healthy control group. Among the 15 elements analyzed, only boron, chromium, zinc, silicon and magnesium were found at clearly detectable concentrations. Colorectal tumour biopsies had significantly higher concentrations of magnesium as compared to adjacent non-tumour or healthy tissues. Zinc concentration followed the same trend but differences were not statistically significant. In addition, concentration of silicon was higher in colorectal cancer tissue than in healthy non-cancer tissue, while chromium was mostly found in adjacent non-tumour tissue.
Conclusion: Magnesium, chromium, zinc and silicon were found in noteworthy concentrations in colorectal tumour. Their potential role in colorectal carcinogenesis should be explored.

1. Introduction

Colorectal cancer accounts for over 9% of all cancer incidences. In the European Union, it is the third most common cancer diagnosis among men and women [1]. This worldwide mortality is approximately half that of the incidence (i.e. about 4.5%). Several risk factors are associated with the incidence of colorectal cancer. Among them, the non-modifiable risks (i.e., those that an individual cannot control) include age and genetic. Hereditary factors, which are involved in 5% of colorectal cancers, are represented by familysusceptibility, familial adenomatous polyposis, and Lynch syndrome. Some digestive diseases are predictive factors of colorectal cancer, including chronic bowel diseases (Crohn's disease and haemorrhagic rectocolitis). However, colorectal cancer is also considered to be an environmental disease. Thus, several lifestyle-related factors have also been identified, such as nutritional practices, physical activity and obesity, cigarette smoking, and heavy alcohol consumption. Although numerous environmental risk factors may play an important role, those risk factors identified do not fully explain the frequency of colorectal cancer. Occupational studies have tentatively linked colorectal cancer to asbestos [2,3] and to metal dust [4]. Hence, a new field is receiving increasing attention, namely the study of environmental risk factors associated with the presence of chemical elements [5,6], such as exogenous micro- and nanoparticles [7].

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Some elements are essential for numerous metabolic and physiological processes in the body, as well as the synthesis and structural stabilization of enzymes, proteins, and nucleic acids. For example, Zn is essential for the functioning of more than 300 enzymes [8] and elements such as Cu, Fe, Mg, Ni, and Zn form compounds with proteins that have important catalytic functions. Cancer is fundamentally a disease of tissue growth regulation. During tumorigenesis, some metal ions (e.g., Fe, Mg, Ni, Zn) induce binding competition with chromatin (e.g., DNA, histones, transcription factors, DNA repair enzymes) and other regulatory molecules that first give rise to tumours and then are responsible for controlling tumour growth. In addition, many studies have identified metal-induced carcinogenicity, demonstrating strong oxidative stress induced by elements such as Cd, Co, Cr, Cu, Fe, and Ni. Free radicals are highly reactive chemical species that have the potential to harm cells, including damage that may lead to cancer. In fact, the generation of reactive oxygen species (ROS) can cause DNA damage and enhance lipid peroxidation. The first-line antioxidant defense of the organism interacts with and neutralizes the effects of ROS, thus preventing them from causing damage. Antioxidants are also known as "ROS scavengers." However, many antioxidant enzymes, such as superoxide dismutases, have an active metal centre (Cu, Mn, or Zn). So, the metal element concentrations in tissues can have a strong impact both on the oxidative stress and the antioxidant defense system. In other words, an imbalance in the optimum level of certain elements may lead to colon carcinogenesis due to a poor redox regulation. However, data on the elemental concentration ranges in human colorectal biopsies are scarce and fragmentary. As a result, a main challenge is to improve our knowledge of the elemental fingerprint of tumour tissue compared to non-cancer tissue.

The aim of this study was to evaluate the element distribution in colorectal adenocarcinoma biopsies, adjacent non-tumour tissues, and healthy controls. To do so, we investigated the elemental distribution in colorectal biopsies of cancer patients (i.e., in tumour and adjacent non-tumour tissues) compared to those of healthy controls (occlusion or ischaemic colons). We used the same patients for evaluating the concentrations of various elements (Al, B, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, Si, Ti, V, and Zn) in cancer and non-cancer tissue, this way allowing for a reliable comparison with potential biases such as genetic, environmental, or dietary factors being eliminated.

2. Methods

2.1. Patients

This case–control study was performed in the Digestive Surgery and Gastroenterological Departments of the University Hospital of Saint Etienne (France). To avoid the bias of selection, the clinical protocol was proposed to each collectomized patient between March 2011 and June 2013 at the University Hospital of Saint Etienne. Patients were considered for the study if they were at least 18 years old and an affiliated member of the social security system at the time of the surgical procedure, which included total or partial colectomy. Before inclusion, each patient provided written informed consent. The exclusion criteria were refused consent, patients under guardianship, rectal cancers initially irradiated, adenomatous polyposis, inflammatory bowel disease (i.e., Crohn's disease or haemorrhagic rectocolitis), familial adenomatous polyposis, or Lynch syndrome. Data on patients' treatment were collected.

A total of 104 patients were included. The colorectal cancer group comprised 76 patients. A first colorectal biopsy was performed within the tumour area, and a second was performed within adjacent non-tumour (i.e., peritumor) tissues. Patients were eligible for inclusion in the colorectal cancer group if the histological findings confirmed the cancer as an adenocarcinoma, regardless of the tumour staging. The control group comprised 28 patients with occlusion or ischaemic colons. Patients were eligible for inclusion in the control group if they underwent a colectomy for any aetiology except colorectal cancer or inflammatory bowel diseases. Histological analysis of all colorectal biopsies included in this study was performed at the Anatomopathology Department.

2.2. Sampling and conservation of samples

The colorectal biopsies were transported to the Anatomopathology Department in sterile packaging without being fixed. The colectomies were put on a sterile sheet and manipulated with sterile and disposables scissors, pliers, and bistouries. Pieces of colectomy were opened following the central line of haustrations. In case of tumour invasion of this line, the tumour was avoided in order not to disseminate tumour cells remotely. For the patients in the colorectal cancer group, we performed a mucosal biopsy (about 1 cm^2 and 0.2 g) in the tumour area and in the adjacent non-tumour tissue; in the latter case, the biopsy was taken at least 10 cm away from the tumour tissue area. For the control group, one mucosal colic biopsy (about 1 cm^2 and 0.2 g) was performed. Consequently, three groups of biopsies were collected: (1) colorectal biopsies from tumour tissue, (2) colorectal biopsies from adjacent non-tumour tissue, and (3) colorectal biopsies from non-cancer patients (i.e., healthy tissue). Colorectal biopsies were rinsed thoroughly with sterile water, placed into sterile standard containers, and snap-frozen with liquid nitrogen at −196 °C until later analysis.

2.3. Element analysis

To avoid classification bias, the multi-element analysis was performed in a blind manner by a technician. Acid digestion procedures are necessary to quantify elements in organic samples using atomic spectrometric methods such as inductively coupled plasma atomic emission spectrometry (ICP-AES). This mineralization step allows complete transfer of the analytes into solution, so that they can be dosed in liquid form. As a result, the goal of the digestion procedure is to obtain the complete dissolution of the analytes and complete decomposition of the organic matrix, while avoiding loss or contamination of analytes.

Each colorectal sample was thawed and weighed. For the acid digestion, biopsies were placed individually in a small Petri dish previously rinsed with 70% alcohol. First, a mechanical dissolution was performed by dilacerating biopsies with disposable sterile scalpels. Then 3 M HCl was introduced, and samples were incubated overnight under soft agitation. The next day, the supernatant and remaining tissue debris were placed into Salivex tubes for ICP-AES analysis. Tubes were heated on a hotplate (99 °C) until all the liquid had evaporated. Finally, samples were put into suspension in 10 ml of 2 M HCl and preserved at 4 °C until ICP-AES analysis. There was no further analytical dilution.

ICP-AES was used to quantify the concentrations of minor elements in each colorectal biopsy. Based on previously published data [6] we investigated the presence of 15 elements. Each element has a specific limit of detection (LoD, concentration of elements expressed in parts per billion i.e. in ng per g of wet tissue): aluminium (LoD of 14.1 ng/g), boron (LoD of 1.5 ng/g), cadmium (LoD of 1.3 ng/g), chromium (LoD of 2.8 ng/g), copper (LoD of 2.4 ng/g), iron (LoD of 1 ng/g), magnesium (LoD of 0.1 ng/g), manganese (LoD of 0.2 ng/g), nickel (LoD of 4.1 ng/g), lead (LoD of 25.1 ng/g), selenium (LoD of 58.3 ng/g), silicon (LoD of 4.4 ng/g), titanium (LoD of 0.6 ng/g), vanadium (LoD of 3.6 ng/g), and zinc (LoD of 0.8 ng/g). Results of concentration of minor element in each colorectal biopsy