Case Report

Molecular Imaging of Cancer Stem Cells for Assessment of Rectal Cancer

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ABSTRACT: Rectal cancer is one of the most common diagnosed malignancies in the world. We present the case of a 54 years old patient, diagnosed with adenocarcinoma of the rectum and unsuccessful treatment. The possible involvement of cancer stem cells in tumor relapse and treatment failure represents the motivation behind an extensive imaging evaluation. The aim of our case report was to assess the outcome of rectal cancer assessment using standard and state-of-the-art techniques, including evaluation of colorectal cancer stem cells. Our results suggest concordant outcomes of modern versus gold standard techniques but further studies are necessary to evaluate the utility during routine clinical work-up.

KEYWORDS: *rectal cancer, cancer stem cells, confocal laser endomicroscopy*

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide, and the second leading type of cancer in Romania, in terms of new cases and deaths, in both females and males (1,2). Of all CRC, rectal cancer has an incidence of approximately 35% in the European Union (3). The number of newly diagnosed cases is expected to increase in parallel with the aging of the population, development of screening programs, emerging imaging techniques and a better understanding of recent carcinogenesis theories. In this context, molecular profiling and specific testing represents the preconditions for personalized medicine, a goal to achieve.

The concept and discovery of cancerous stem cells (CSCs) are relatively new and consider stem cells to be progenitors of cancer requiring the accumulation of genetic/epigenetic aberrations. Alterations as uncontrolled replication and deregulated differentiation will cause the changeover to CSCs, caring the potential of tumor initiation and maintenance. It is important to describe these cells in order to better understand the mechanisms of resistance, develop targeted therapies, as well as anticipate outcome and response to treatment. CSCs can be identified by specific antibodies directed at the protein markers present on the cell surface, markers as: CD133, CD166, CD44, CD24, EpCAM (ESA), beta1 integrin-CD29, Lgr5, Msi-1, DCAMLK, ALDH-1or EphB receptors (4).

Currently the evaluation of CSCs expression patterns can be performed using different methods, out of which the newest aspects of confocal laser endomicroscopy (CLE), immunohistochemistry (IHC) and molecular biology were selected for this study.

Applying research findings, adapting the guidelines recommendations to each individual patient and predicting the treatment response represent a key challenge for clinicians. The aim of our study was to assess the outcome of multidisciplinary CRC evaluation combining standard and state of the art techniques.

Case report

We present the case of a 54-year-old Caucasian man, who presented to his primary care provider with the complaints of unintended weight loss (25 kg during the previous year), loss of appetite, alternating constipation and diarrhea, fatigue. The patient was referred to our service for further diagnosis, treatment and follow-up. No relevant information was related regarding his medical, family or social history. At the physical examination the patient appeared as a pale, thin man in no acute distress, anicteric sclera with pale conjunctiva, no palpable adenopathy, normal cardiac and pulmonary auscultation, soft abdomen without pain, distention or tenderness, normal bowel sounds present in all quadrants, no hepatosplenomegaly. Vital signs were as follows: blood pressure 125/80 mmHg, heart rate 68 bpm, respiratory rate 21 breaths/minute, temperature 37°C. Laboratory findings: Laboratory studies indicate a hemoglobin level of 12.1 g/dL and a mean

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corpuscular volume of 93 fL, CEA 4,72 mg/ml, CA 19.9 6,6 U/ml. The colonoscopy evaluation revealed a fragile, bleeding tumor and 1 cm polyp situated near the tumor, 19 cm away from the external anal sphincter. Fresh biopsies from tumor and normal tissue were harvested and referred for pathology, IHC, molecular biology and CLE evaluation. Pathology result described the presence of adenocarcinoma G1-G2, with ulceration and invasive character and mucosa with chronic inflammatory infiltrate. Abdominal ultrasound was negative, CT of the chest and abdomen were also without any pathological changes while the pelvic area showed focal, irregular thickening of the rectum wall, 1,8 cm up to 2,8 cm, iodophil, with the infiltration of the perirectal fat. Contrast enhanced endoscopic ultrasonography (CE-EUS) was performed with a linear echoendoscope showing a hypoechoic mass situated from 5 cm to aprox 15 cm away from the external anal sphincter. The tumor was circumferential, with an inhomogenous pattern, infiltrating all the layers of the rectal wall (T3) which was thickened up to 17 mm. In several sections it seemed to present direct contact with the prostate and outlining a cleavage plane. The power Doppler examination was further performed showing native intratumoral signals, enhanced after the administration of contrast (Sonovue 4,8 ml). Contrast-enhanced examination with a low-mechanical index (0.2) mode showed uptake of the contrast agent during the arterial phase, excepting few areas near the rectal lumen (interpreted as necrosis zones). Two peritumoral lymph nodes were described as round-oval, hypoechoic with the diameter of 5 mm respectively 9 mm (N1). After complete diagnosis and staging (rectal adenocarcinoma, Stage IIIB-T3N1M0, G1-G2 histologic grade) patient was addressed to the oncology department for radiochemotherapy. Radiation treatment was directed to the pelvis region using the box technique with 10MV photons up to a total dose of 45 Gy, 1,8 Gy/day in 25 fractions. Patient was scheduled for simultaneous chemotherapy with a 5-FU based regimen concomitant with oxaliplatin and bortezomid. After finishing the oncologic treatment and prior to the radical surgery, patient was reevaluated by CE-EUS and no major improvements regarding tumor characteristics were observed (size, local extension, lymph nodes, power Doppler signal, contrast enhancement were all similar to the assessment prior to radiochemotherapy).

The CLE evaluation was performed using a dedicated CLE system which integrates a miniature confocal microscope into the distal tip of a conventional flexible endoscope (EC-3870 CIFK, Pentax, Tokyo, Japan). Immediately after collecting it, the biopsies were marked with fluorescently labeled anti-CD166 and anti-CD 133, placed in direct contact with the distal tip and controlled by the user during the examination for optimal imaging contrast. During the scanning, the laser delivers an excitation wavelength of 488 nm with a maximum laser power output of ≤1 mW at the surface. A series of optical slices spanning 250 mm inside the tissue were collected and analyzed using Image J- image processing software (National Institutes of Health, USA). Images with the strongest fluorescent signal and a good display of the tissue and CSCs were selected in order to be counted.

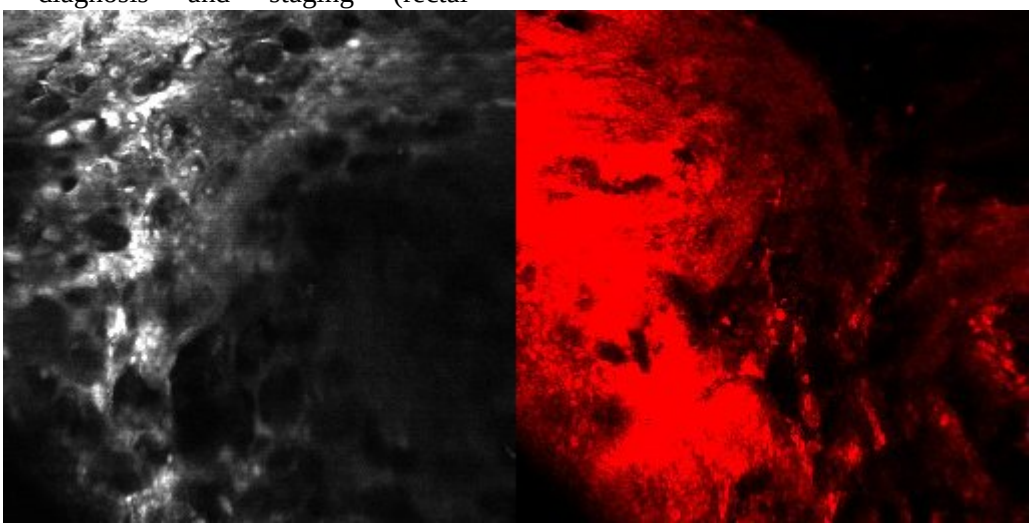

Fig.1. Confocal laser endomicroscopy image of normal tissue marked with fluorescently labeled anti- CD133 antibody

Regarding the CLE evaluation of the CD 133 biomarker, the most relevant 10 images were selected and the cell count revealed 16,3±3,87 cells/slide for the tumoral tissue and 37,45±24,36 cells/slide for the normal tissue ($p<0,01$).(Fig.1, 2) Biopsies marked with anti-CD166 antibody showed no signal, the images being impossible to evaluate. In the same session, acriflavine staining of bioptic material was performed, being one of the specific nuclei staining agents. Acriflavine solution was sprayed topically and the material was scanned and analized according to the already described protocol, observing the presence of nuclei and a high concentration of possible fibrin. (Fig.3)

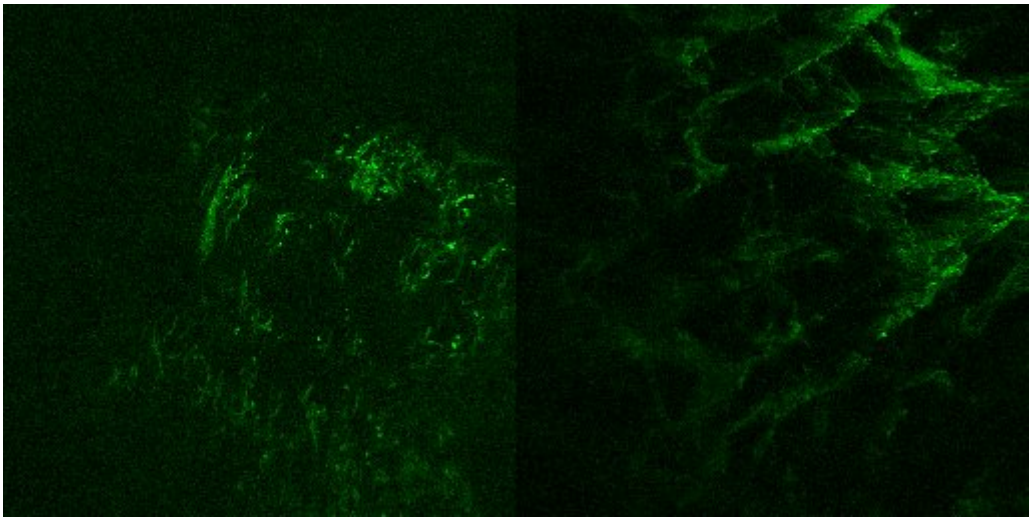

Fig.2. Confocal laser endomicroscopy image of tumor tissue marked with fluorescently labeled anti- CD133 antibody

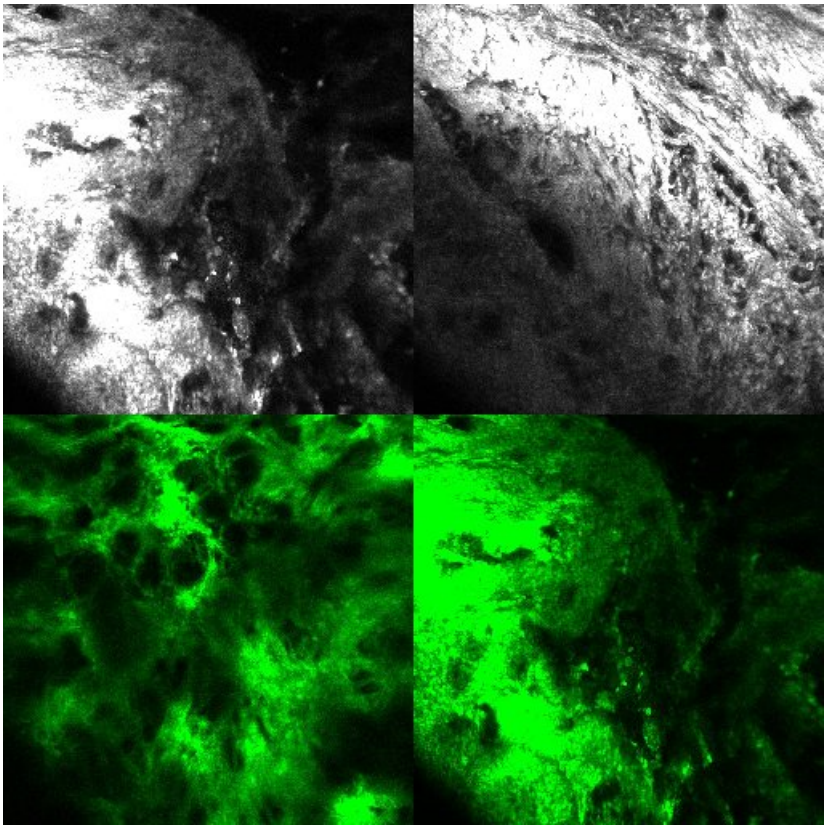

Fig.3. Confocal laser endomicroscopy image of acriflavine staining revealing nuclei and fibrin

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Regarding molecular biology evaluation a two step quantitative real-time polymerase chain reaction (qRT-PCR) method was used for measuring PROM1/CD133, ALCAM/CD166 gene activation level in paired samples. The complementary DNA (cDNA) synthesized by reverse-transcription was quantified using TaqMan technology. Total PROM1 was higher expressed in peritumoral normal tissue, for ALCAM the difference between paired samples was biological insignificant.

Immunohistochemistry evaluation: paired biopsies were investigated by enzymatic and multiple fluorescence IHC for their CD133 and CD166 expression. Triple immunolabeled CD133-CD166-Ki-67(DAPI) slides were analyzed utilizing a Nikon Eclipse 90i motorized microscope (Q-Imaging, Surrey, BC Canada), together with the Image ProPlus AMS analysis software (Media Cybernetics, Bethesda, MD, USA). CD133 expression correlated with that of CD166, the colocalization being more obvious especially at the cells membrane level along the entire spectrum of lesions. For both markers the tumor specimens presented heterogenous staining pattern. CD133 staining was present in the cytoplasm and on the apical plasma membrane of cells within tumor gland like entities. In normal tissue the marker had a weak expression in the cytoplasm of enterocytes. In tumor tissue CD166 showed both citoplasmic and membranous patterns, with a more pronounced membranous expression while in normal tissue it was prevalent as a membranous staining of the cells forming the base of the crypts.

Discussions

In the present study, we reported the case of a patient diagnosed with rectal adenocarcinoma on which conventional radiochemotherapy failed. The uniqueness of the case lays in the use of multiple state-of-the-art techniques and methods in evaluating the current theories regarding carcinogenesis, tumor development and drug resistance.

The best option for first-line treatment in advanced CRC is still rather complex and unclear thus describing predictive markers might represent the much needed progress in the choice of adequate, individualized biological therapy.

One of the reasons behind treatment failure in CRC is the existence of drug-resistant colon CSCs (5). Actually, traditional treatment has a broad cytotoxic activity being designed to kill as many cancer cells within a tumor and consequently to induce the tumor regression. Not targeting specifically the CSCs, the bulk of spared cells can eventually regenerate the tumor and even lead to relapses. All in all, the failure of chemotherapy might be related to its limited capacity of affecting the rapidly growing cells to divide, without addressing the CSCs. Furthermore, a treatment targeting directly the CSCs might not determine express reduction of the tumor size but might acquire long term disease abolishment by depleting the tumors self-renewal and growth potential (6-9).

To overcome the obstacle of the currently unpredictable inter-individual variability in the therapy outcome, concentrated research efforts must focus on elucidating the complex mechanisms behind CRC tumorigenesis. Thus, we aimed to investigate the CSCs and the expression of their targeted markers through CLE, IHC and qRT-PCR. While qRT-PCR is considered the gold-standard technique for measuring gene expression (10), CLE is a novel endoscopic imaging technology that enables real time histological examination of the gastrointestinal mucosa, with a high sensitivity and specificity, being proposed as an alternative endoscopic method to distinguish neoplastic from non-neoplastic lesions (11). The correspondence between CLE and molecular expression pattern of CD 133 and CD166 biomarkers, which was observed in our case, could set a pathway for future individualized therapeutic approach, targeting specific cellular subgroups from a heterogeneous tumor.

Conclusion: The use of modern techniques for evaluating the expression pattern of specific CRC biomarkers offer concordant outcomes with the conventional techniques. The methods could be transcribed into a framework suitable for optimizing prospective therapies, addressing the CSCs subpopulations in order to reduce CRC treatment failure.

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