

Morphological and Immunohistochemical Criteria of Tissue Response to Radiotherapy in Rectal Cancer

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Rezumat

Obiectivarea morfologică și imunohistochimică a răspunsului tisular la radioterapie în cancerul de rect

Premize/Scopuri: În contextul în care tumorile de rect răspund în diverse grade la radioterapie, se impune necesitatea de a estima de la momentul biopsiei diagnostice capacitatea de răspuns a tumorii respective la iradiere.

Material și metodă: Am examinat histologic și imunohistochimic țesuturi provenind de la 52 de pacienți cu adenocarcinoame rectale.

Rezultate: Dintre parametrii studiați, cei care au prezentat o variație semnificativă din punct de vedere statistic ($p < 0.05$) cu radioterapia au fost: reacția coloidală ($p = 0,001$), EGFR în tumoră ($p = 0,00045$), EGFR în epiteliul normal ($p = 0,0017$), VEGF în tumoră ($p = 0,0132$) și VEGF în stroma tumorală ($p = 0,030$).

Concluzii: Rezultatele se aliniază cu cele din literatura curentă consultată referitor la variația cu radioterapia a markerilor EGFR și VEGF, studiul nostru individualizându-se prin

observația conform căreia stroma normală în cazul tumorilor de rect reacționează și ea la radioterapie, uneori mai agresiv chiar decât tumora însăși, mai ales la nivelul filetelor nervoase și a fibrelor musculare.

Cuvinte cheie: cancer de rect, radioterapie, EGFR, VEGF

Abstract

Aim: Given the context that rectal tumours respond to a certain degree to radiotherapy, a necessity arises for estimating a tumour's capacity to react to radiation from the very moment of diagnostic biopsy.

Material and Methods: We have histologically and immunohistochemically analysed tissues coming from 52 patients with rectal adenocarcinomas.

Results: Of the studied parameters, the ones presenting significant variation under radiotherapy in terms of statistics ($p < 0.05$) were: colloid type ($p = 0.001$), EGFR in the tumour ($p = 0.00045$), EGFR in the normal epithelium ($p = 0.0017$), VEGF in the tumour ($p = 0.0132$) and VEGF in the tumour stroma ($p = 0.030$).

Conclusions: Our study follows the same trends as the medical literature we have consulted regarding the variation of EGFR and VEGF with radiotherapy, and the distinct note of our study relies in the observation that normal stroma in case of rectal

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tumors also reacts to radiotherapy, sometimes more aggressively than the tumor itself, especially in which concerns the nerve and muscle fibers.

Key words: rectal cancer, radiotherapy, EGFR, VEGF

Introduction

Current studies and protocols regarding the optimal treatment of rectal cancer involve radiotherapy and chemotherapy as adjuvant and neo-adjuvant therapies. (1)

Preoperative radiotherapy is indicated for rectal tumours stages II and III (2).

In 1996, Bazzetti described 5 degrees of postradiation tumour regression, varying between maximal response (complete regression – the tumour can no longer be histologically identified after radiotherapy) and minimal response (no regression – the tumour is in no way influenced by the radiotherapy) (3). According to the degree of variation of the postradic reaction, a need to estimate the tumor s capacity to respond to radiotherapy from the moment of the diagnostic biopsy becomes obvious, having in mind mostly patients that would not benefit from radiotherapy and would have their surgical procedure postponed uselessly.

Material and Methods

We conducted a retrospective study on 52 patients with rectal adenocarcinomas. The group consists of such a small number of patients because the immunohistochemistry exam is not routinely performed pre- and postoperatively. Paraffin blocks of tissues from rectal tumours, taken both pre- and postirradiation, were examined from the histological and immunohistochemical point of view. The group was comprised of 26 females and 26 males. The abbreviations used in the following sections are: RIB for tissues before radiotherapy, RIC for tissues after radiotherapy, OS for overall survival, DFS for disease free survival, EGFR for epidermal growth factor receptor, VEGF for vascular endothelial growth factor (see *Graph 1*)

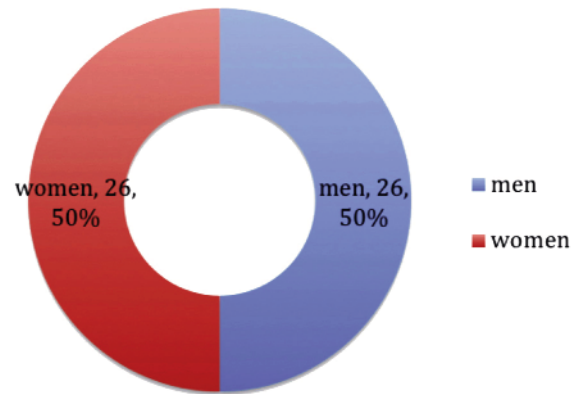
Cell type, desmoplastic reaction, colloid type and cell atypia were studied comparatively before and after radiation, from a morphological point of view.

In terms of immunohistochemistry, the following markers were examined: EGFR in the tumour, EGFR in the tumour stroma, EGFR in the normal epithelium, EGFR in the normal stroma. A chi test was performed for variations of each of the parameters described, and the corresponding p values were noted. Depending on the p value ($p < 0.05$) we determined whether the variations were influenced in a statistically significant manner by radiotherapy or not.

1) The first parameter analysed was cell type: cylindrical /cubic, both before and after radiotherapy.

Cell shape and architecture are determined by cell-extracellular matrix interactions and have profound effects

Patient distribution by sex



Graph 1. Patient distribution by sex was equal between the two sexes, with 26 patients each

on cellular behaviour, chromatin condensation, and tumour cell resistance to radiotherapy and chemotherapy.

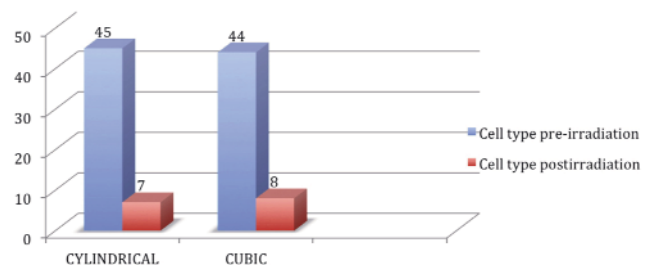
Transformation from cylindrical to cubic: Oncocytic change in this particular clinical context occurs as a reflection of cytotoxic damage or cellular hypoxia induced by chemoradiation resulting in degeneration of the cell and the oncocytic phenotype.

Cell type distribution (45 cylindrical type ADKs and 7 cubic type ADKs) before and after radiotherapy (44 cylindrical and 8 cubic) was not relevant from a statistical point of view (chi test =0.78016 and $p=0.96$) (see *Graph 2*). We present as observation the following aspects: in our group, 2.22% of cylindrical cells transformed into cubic cells (1 out of 45) and 100% of cubic cells (7 out of 7) turned into cylindrical cells.

2) The second morphological parameter studied was the desmoplastic reaction.

Desmoplastic reaction has been defined as being associated with some tumours and is characterized by the pervasive growth of dense fibrous tissue around the tumour. This reaction makes it difficult to distinguish between spiculation in the perirectal fat caused by fibrosis alone from that caused by fibrous tissue that contains tumour cells. Cancer associated with a reactive stroma is typically diagnostic of poor prognosis.

Cell type distribution before and after radiotherapy



Graph 2. Cell type distribution before and after radiotherapy was predominantly cylindrical before radiotherapy and cubic after radiotherapy

With regard to the desmoplastic reaction we can mention that it also did not present statistical signification, with values of chi test=0.276 and p=0.218. Similar to the previous example, we present our results for descriptive purposes: a grade 0, 1 and 2 desmoplastic reaction was encountered pre- and postradiotherapy according to the RIB-preradiotherapy and RIC-postradiotherapy table data. (see Graph 3)

3) The third parameter studied was cell atypia, also of no statistical value (chi test 0.899 and p 0.910), see Graph 4.

Atypical cellular features were studied as they seem to be directly proportionally associated with an increased degree of severity of the neoplastic disease.

The individual tumour cells in treated rectal cancer may show marked nuclear atypia. Marked cytoplasmic eosinophilia, often in association with nuclear atypia is the most commonly seen type of cytologic alteration in tumours remaining in the intestine after radiation.

4) colloid type was the sole morphological parameter analysed to present statistical value, with chi test = 0.003 and p=0,001 (see Graph 5).

Mucinous adenocarcinoma is characterised by abundant extracellular mucin produced by tumour cells. By definition, a 50% or greater mucinous component is required for the designation of mucinous colorectal carcinoma. The prognostic significance of mucinous carcinoma is controversial, many authors associating it with a poor prognosis to chemoradiotherapy.

5) EGFR (epidermal growth factor receptor) exists on the cell surface as a transmembrane glycoprotein, a member of the protein kinase superfamily.

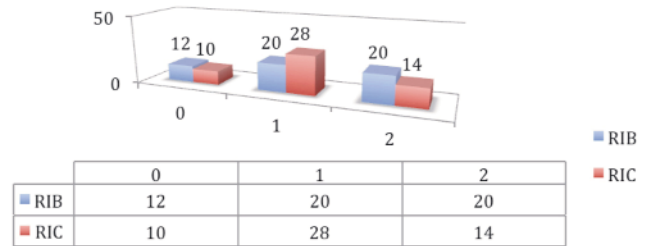
This protein is a receptor for members of the epidermal growth factor family and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α (TGF α). Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers. The identification of EGFR as an oncogene has led to the development of anticancer therapeutics directed against EGFR, including gefitinib and erlotinib for lung cancer, and cetuximab for colon cancer.

Current findings have demonstrated that alterations in tumour stromal pathways, including the EGFR pathways, are associated with, and may contribute to, resistance to VEGF inhibitors and that targeting these pathways may improve therapeutic efficacy. Understanding stromal signalling may be critical for developing biomarkers for angiogenesis inhibitors and improving combination regimens and this is the reason why we chose to study EGFR and VEGF both at the level of the tumour and at the level of the stroma, and the grades are given according to appreciation of two independent examining doctors regarding the amount of EGFR and VEGF in the tissue studied.

EGFR in the tumour increased, on average, after radiotherapy by one unit (chi test 0.00156, p=0.00045, result bearing statistical significance). (See Graph 6 and Fig. 1)

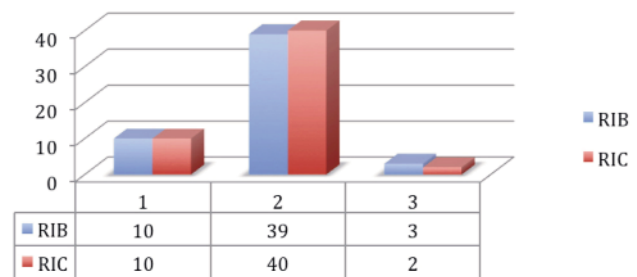
6) EGFR in the tumour stroma presented a tendency to decrease, with chi test 0.32523 and p=0.169 (83.1% probability for the null hypothesis to be false).

Distribution of desmoplastic reaction before and after radiotherapy



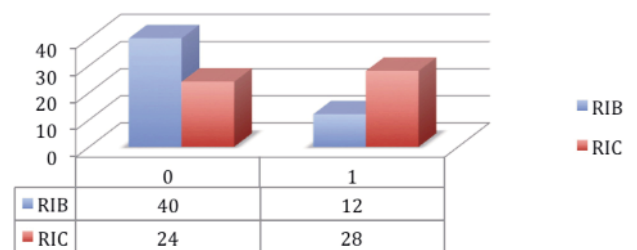
Graph 3. The distribution of desmoplastic reaction before and after radiotherapy: if before radiotherapy the predominant desmoplastic reactions were of grades 1 and 2, after radiotherapy the predominant desmoplastic reaction becomes 1

Distribution of atypia before and after radiotherapy



Graph 4. The variation of the distribution of atypia before and after radiotherapy has kept the same type 2 as the main type of atypia

Distribution of the colloid response before and after radiotherapy

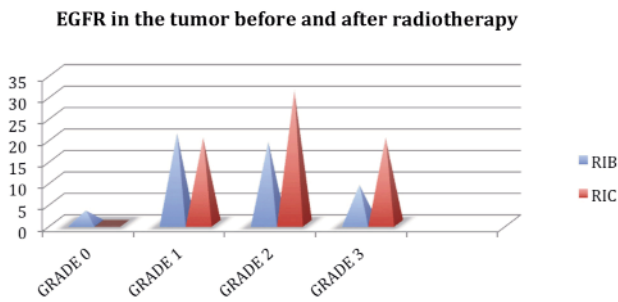


Graph 5. The variation in the distribution of the colloid response before and after radiotherapy changes consistently from dominant 0 to dominant 1

78% of tissues with EGFR 0 in the tumour stroma maintain their EGFR 0 status, 22% become EGFR 1. (See Graph 7)

7) Also, EGFR in the normal epithelium presented an increase due to radiotherapy, with chi test 0.053 and p=0.0017 (see Graph 8). Another observation we would like to draw attention to is that EGFR in the normal stroma is increasingly expressed in the smooth muscle and in nerve fibers, normal tissues which appear to be modified as a result of irradiation, with Chi 0.0002 and p=0.02.

8) Tumour growth and metastasis are dependent on the



Graph 6. The variation with radiotherapy of EGFR in the tumor: the tissues before radiotherapy had similar numbers of degrees 1 and 2, and after radiotherapy degree 2 was the main degree found among the tissues examined

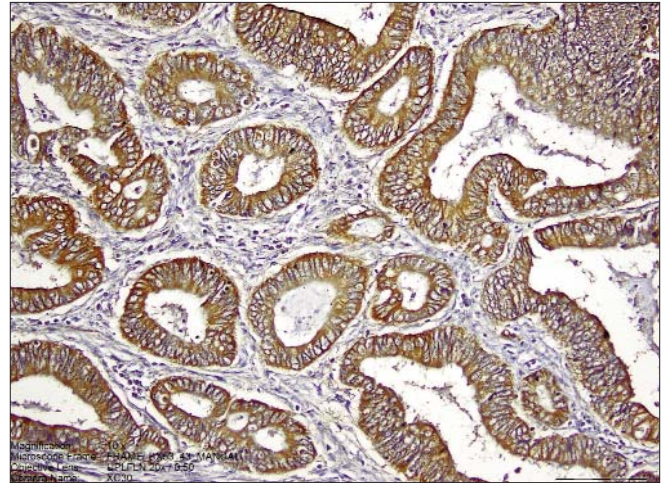
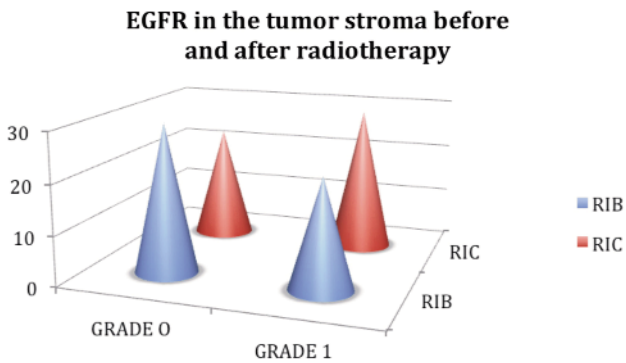
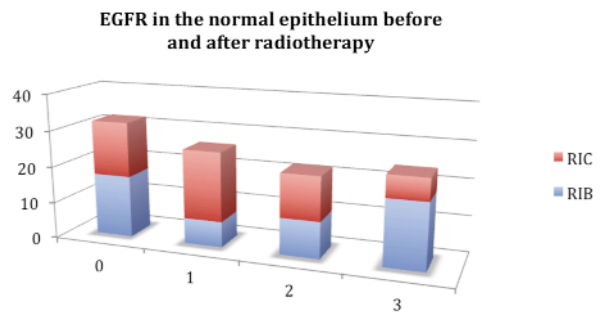


Figure 1. The areas in brown show EGFR in the tissues after radiotherapy



Graph 7. The variation with radiotherapy of EGFR in the tumor stroma



Graph 8. EGFR in the normal epithelium before and after radiotherapy

formation of a vascular supply, i.e., angiogenesis.

Most therapeutic efforts directed toward inhibiting the angiogenic process for the treatment of cancer have focused on the VEGF pathway.

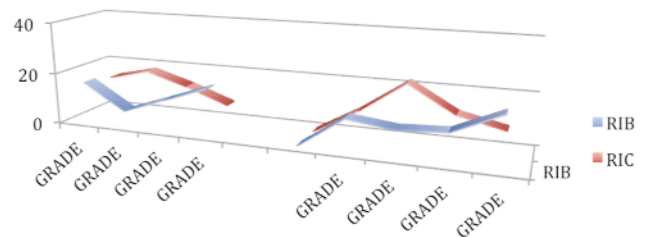
VEGF variation in the tumour showed statistical relevance, after a chi test=0.0456 and $p=0.0132$, and VEGF in the tumour stroma varied under radiotherapy with a chi test of 0.0094 and $p=0.030$; for a comparison of the two, see Graph 9. For the aspect of VEGF in the tissues, see Fig. 2.

Discussions

1) The increase in colloid type from degree 0 to 1 in the majority of cases would lead us to the logical idea that radiotherapy either induces or aggravates the colloid type. The literature consulted indicated that cancers which seem to be induced by radiotherapy present features predominantly colloidal (4).

2) The increase in EGFR under radiotherapy in the majority of patients was in accordance with the results of most of the studies consulted. However, these results might bear negative clinical implications, for patients with an increase in EGFR postradiotherapy had, according to some international studies, a lower global survival rate and a shorter disease free interval. (5) Also, our note regarding the significant increase in

Variation with radiotherapy of the values of the VEGF in the tumor and in the tumor stroma



Graph 9. Comparison between the VEGF values before and after radiotherapy at the tumor level (first pile to the left of the viewer) and at the level of the tumor stroma (pile at the right of the viewer)

EGFR expression in the normal stroma at the levels of smooth muscles and nerve fibres involves an attentive research on studies connecting this aspect to the survival rates and aggressiveness of the disease. Consulting pubmed archives led to the identification of a study correlating the reaction of the stroma, rather than the tumour, to radiotherapy in oral neoplastic diseases with the survival rate (the stromal reaction to radiotherapy appears to be a stronger statistically predictive factor

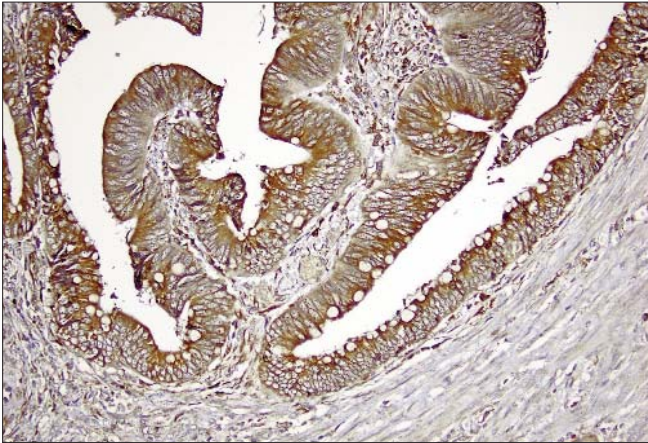


Figure 2. The areas in brown show VEGF at the level of the tumor after radiotherapy

than the tumour's reaction) (6).

3) VEGF increase both in the tumour and the tumour stroma, as a result of radiotherapy, was also proved by other studies as well, indicating a phenomenon according to which the increase in VEGF is related to an increased nodal involvement, to deeper tumour invasiveness, to a higher rate of radiation proctitis (7) and to the TNM stage (8).

4) The most important limitation of our study is the low number of cases, especially in situations in which insufficient tissues for the study were available, with a negative rather than positive effect on statistics.

5) As future perspectives, along with increasing the number of cases for the studied markers, it would be interesting to determine their correlation with OS global survival rate and with the DFS disease free period.

Conclusions

- 1) The following morphological factors studied in our group were of no significant statistical value, therefore ineffective in demonstrating or quantifying the influence of radiotherapy on the tumours: Cell type, desmoplastic reaction and cell atypia. On the other hand, colloid type suffered an important change under radiotherapy, turning in the majority of cases from 0 degree to 1 degree.
- 2) Other statistically significant markers were EGFR in the tumour, EGFR in the tumour stroma, EGFR in the normal epithelium, the latter increasing as a result of radiotherapy. Moreover, EGFR was increasingly expressed in smooth muscle fibres and nerve fibres, proving the involvement of normal stroma reaction to radiotherapy. (9,10)
- 3) Another marker modified as a result of radiation was VEGF in the tumour and VEGF in the tumour stroma. (11,12).

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Authors contribution

Authors marked 3, 4, 5, 6, 7 provided part of the cases, the clinical material studied and the first 6 points described in Material and Methods. Authors marked 2 attended the cases directed to their services and established which markers should be studied and which tissues out of the ones available should be tested, and provided the specialized pictures. Author number 1, Sinziana Ionescu ensured the integration of the clinical, paraclinical and theoretical information regarding the cases, and redacted the theoretical support for this article, while the second author, Professor Eugen Bratucu, corrected the entire article, modified the charts and added the Discussion and Results sections."

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