MMP7 Modulation by Short- and Long-term Radiotherapy in Patients with Rectal Cancer

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Abstract. Background/Aim: Matrix metalloproteinase 7 (MMP7) expression is highly associated with colorectal cancer and modulates tumour growth and invasion. Radiation injury induces inflammation with increases in MMP7 and in transforming growth factor beta (TGFβ). The aim of this study was to investigate the effect on MMP7 and TGFβ expression in patients with rectal cancer undergoing different regimens of neoadjuvant radiotherapy (RT). Patients and Methods: We studied 53 patients in three RT treatment groups receiving RT of 25 Gy, long-term RT 50 Gy and controls receiving no RT. Three biopsies were obtained from each patient during the treatments: before RT, after RT and after surgery. Tissue samples were formalin fixed, paraffin embedded and tissue microarrays were constructed and stained for MMP7 and TGFβ. Mann–Whitney U-tests and Wilcoxon Z-tests were used to determine differences between patients before and after RT, and after surgery, as well as between the RT groups. Results: In all three patient groups, increases of MMP7 and TGFβ expression were observed after surgery. MMP7 expression was significantly increased in patients receiving short-term RT but TGFβ expression was not affected by RT. Conclusion: 50 Gy Irradiation of rectal cancer gives less tumour activation of MMP7, whilst it is up-regulated by 25 Gy and surgery regardless of RT.

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MMPs are induced by interactions not only between cells and the ECM but also by cytokines and growth factors. The ECM is remodelled by ionizing radiation which causes extensive oxidative damage at the cellular level and of numerous enzymes, for example the multifunctional inflammatory cytokine transforming growth factor beta (TGFβ) and MMPs (9).

Malignant epithelial cells are unique in expressing matrix metalloproteinase 7 (MMP7), known as matrilysin, a protease that increases in metastatic colorectal cancer (9). In a murine model of radiation-induced enteropathy, both MMP7 and TGFβ were up-regulated by irradiation (11). RT has also been found to induce an elevation of MMP7 in humans (9, 12). During wound healing, activation of MMPs is required in several of the steps leading to restoration of tissue integrity.

The aim of this prospective study was to investigate if different RT regimens with standard surgical treatment affect MMP7 and TGFβ expression in patients with rectal cancer. 

Patients and Methods

Patients. This study was a prospective descriptive study where originally 77 patients diagnosed with rectal cancer were treated at Skåne University Hospital in Malmö, Sweden, between 2004 and 2007. All patients were managed according to the clinical protocol of the Department of Clinical Sciences, Division of Colorectal Surgery, adhering to national guidelines and assessment of the local multidisciplinary treatment board. A total of 24 patients were excluded from the study due to revised pathological diagnosis showing high-grade dysplasia (eight patients), impaired general condition (eight patients), declining to participate (four patients), synchronous colonic tumours (three patients) and for logistical reasons in one patient, with the final cohort therefore comprising 53 patients. Seventeen patients were female (32.1%) and 36 were male (67.9%). The study was approved by the Ethics Committee at Lund University, Sweden (ref 144/2004, amendment 597/2006) and registered at Clinical Trials, ID NCT03151759. Written consent was obtained from each patient after provision of oral and written information. Staging was performed according to the TNM system (13). Exclusion criteria included previous RT to the pelvic region, inflammatory bowel disease, neoadjuvant chemotherapy, as well as ongoing steroid, immunosuppressive or antibiotic therapy.

Three treatment groups were defined: one group received short-term preoperative RT of 25 Gy (5×5 Gy) followed by surgery, 20 patients; another group was treated with long-term preoperative RT of 50 Gy (28×1.8 Gy) followed by surgery, 21 patients; and a control group underwent surgery alone without RT, 12 patients.

Surgical procedures performed were either anterior resection or abdominoperineal resection, by the total mesorectal excision technique. A rigid rectosigmoidoscopy was performed before and after irradiation/before start of surgery. Two-millimeter punch biopsies were obtained from tumour tissue within the irradiated field. Samples were taken on three occasions: at inclusion before RT; after RT prior to start of surgery to eliminate possible effects of surgical trauma and ischaemia on MMP expression; and from the excised specimen. All tissue samples were instantly formalin fixed and paraffin embedded.

Tissue microarrays. Tissue microarray (TMA) was performed in two series: one TMA with 1×1 mm cores taken from biopsies of tumour tissue, and one TMA from the complete surgical specimens through which 2×1 mm cores were drawn from areas comprising tumour. This was performed using a manual arraying device (MTA-1; Beecher Inc., Sun Prairie, WI, USA) and tissue samples were mounted in a recipient block.

Immunohistochemistry and staining evaluation. The TMAs were cut into 4-μm sections pretreated in a DAKO PT-link module using a standard protocol and buffer supplied by the manufacturer (DAKO, Glostrup, Denmark). Thereafter slides were stained in a DAKO Autostainer-plus using the EnVision™ FLEX including Peroxidise-Blocking Reagent (DAKO) with mouse monoclonal antibody to MMP7 (dilution 1:50; Santa Cruz, Dallas, TX, USA) and rabbit polyclonal antibody to TGFβ (dilution 1:200; Abcam, Cambridge, UK). Immunohistochemistry was performed by an automated staining machine (Ventana Medical Systems, Inc., Tucson, AZ, USA).

Three research scientists jointly annotated cytoplastic expression of MMP7 and TGFβ in tumour tissue for each core. Annotation of the absolute percentage of positively stained cells was multiplied by the annotated intensity (scored 0-3) of stained cells, and a mean expression score was subsequently calculated for each patient at each time point. This method was validated earlier (14-16). Discrepant cores were discussed until consensus was reached.

Statistical analysis. Spearman’s Rho and chi-squared tests were used to investigate RT groups and patient characteristics (Table I). Mann–Whitney U-test/Wilcoxon Z-test were used to investigate differences in MMP7 expression between tissue before and after radiotherapy, and after surgery, in the RT subgroups, as well as for the TGFβ analysis. Fishers exact test was used in single case analyses. All statistical analyses were performed using SPSS ver.21.0 (IBM Corp., Armonk, NY, USA); associations/differences with p-values of less than 0.05 were considered significant.

Results

The majority of patients in the different groups were male and younger than 75 years. Disease stages were equally distributed among groups with the exception of stage IV, with a very low number of cases enrolled (Table I).

For the whole cohort, no significant increase of MMP7 or TGFβ was observed after RT, whereas after surgery, MMP7 expression was significantly increased (p<0.001, Z=−6.167) as was that of TFGβ (p<0.001, Z=−6.651) (Figure 1).

In patients treated with short-term RT, MMP7 showed a borderline significant increase after RT compared to baseline (Fishers’ p=0.065, two-tail p=0.025, Z=−2.230), and a significant rise after surgery (Fishers’ p<0.001, Z=−4.147) compared to baseline (Table II). TGFβ was not significantly affected by RT but also significantly increased after surgery (Fishers’ p<0.001, Z=−4.010) (Table III).

In the group treated with long-term RT, both MMP7 and TGFβ were unaffected by RT but increased after surgery (MMP7: Fishers’ p=0.005, Z=−2.870; TGFβ: Fishers’ p<0.001, Z=−3.862).
Expression in patients not treated with RT exhibited a similar trend, with increased MMP7 and TGFβ expression after surgery (MMP7: Fishers’ \( p = 0.002 \), Z=-3.208; TGFβ: Fishers’ \( p < 0.001 \), Z=-3.399).

**Discussion**

The expression of MMP7 m-RNA in humans is highly specific for malignant epithelial cells of colorectal cancer, with it being only weakly expressed in normal colorectal mucosa, with a progressive trend from normal mucosa to cancer. Varied expressions with increasing grade of dysplasia and inflammation have been observed (9, 17-19). In cancer immunology, a clear role of MMP7 and of other MMPs has been shown in tumour growth and invasion (17, 20-25). Increased MMP7 expression in tumour tissue, serum, lymph nodes and peritoneal liquid generally correlates with worse prognosis, a tendency for metastatic disease and reduced overall survival (18, 26-32). This suggests a possible role for MMP7 in the determination of locally advanced cancer in resected specimens, and in staging and planning for eventual adjuvant therapy, attesting to a potential role of MMP7 as prognostic factor and tumour marker (33).
The effect of RT on MMP7 gene in human rectal cancer was first investigated in vivo by Kumar et al., who observed overexpression of MMP7 in radiated rectal cancer tissue compared to nearby irradiated normal rectal tissue (9). Due to the strict correlation between surgery and RT in the current multimodal management of rectal cancer, the effect and the consequent possible modulation of radiation-induced MMP7 overexpression remains a current field of investigation which is still far from complete due to its complex features and to the several molecules and pathways involved.

The expression of MMP7 and of other MMPs is usually the result of specific immunological stimuli due to physiological and pathological processes leading to proliferation and remodelling in tissue growth and differentiation. This is seen in tissue repair and wound healing after inflammation, and after various types of injury and trauma (11, 34-36). In our clinical setting, there was a double factor potentially acting on ECM remodelling since RT alone or surgery alone, or both in combination, clearly affected MMP7 expression. Control cases (no RT) presented significantly higher expression only after surgical resection. In cases irradiated with 50 Gy, the effect of RT itself seemed to not influence MMP7 expression; however, there was a tendency for radiation to reduce MMP7 expression, probably reflecting less radiation injury of the tissue. Only after surgery did these irradiated patients present significantly higher expression of MMP7 comparable to that of the the 25 Gy RT group, which after RT alone already had a significant increase in MMP7 expression before surgery. Expression significantly further increased after surgery. The analysis of these results demonstrated that MMP7 is overexpressed after different types of stimulation. Surgery alone was effective in inducing up-regulation and, in combination with short-term (25 Gy) RT, led to a further overexpression of MMP7 after RT. Higher doses of RT (50 Gy) with surgery delayed by more than a month did not lead to any increases in MMP7 at the time of surgery compared to level observed at baseline. The explanation here might be ascribed to the waiting time after sample collection since according to the neoadjuvant protocol, patients underwent surgery 6-8 weeks after completion of RT, which is sufficient time for the effects of induced overexpression to be lost. Our results for the 50 Gy-treated group, however, may be biased by the low number of patients that it was possible to adequately examine and analyze. This was due to the fragility and poor staining of the specimens from such patients, whereby only five cases were accepted for examination. Given the role of MMP7 in abnormal tissue remodelling after RT injury (9, 25, 29) and its relation to progressive and metastatic colorectal disease, it may be rational to use potential preventive or therapeutic specific tissue inhibitors of MMPs with RT.

Surgical trauma was seen to affect MMP7 expression in all the examined groups. Our observation of the marked

### Table II. Tumour expression of matrix metalloproteinase 7 (MMP7) before and after treatment. Mean rank is used to approximate relation between the MMP7 at treatment times.

| Group       | Time point | n  | MMP7 mean rank | p-Value* | Z-value* |
|-------------|------------|----|----------------|----------|----------|
| Long-term RT | Baseline   | 10 | 8.05           |          |          |
|             | Before surgery | 9  | 16.56         | 0.065    | −2.230   |
|             | After surgery | 16 | 25.03         | <0.00001 | −4.147   |
| Short-term RT | Baseline   | 13 | 12.92         |          |          |
|             | Before surgery | 5  | 9.00          | 0.336    | −1.353   |
|             | After surgery | 14 | 22.50         | 0.005    | −2.870   |
| No RT       | Baseline   | 9  | 10.00         |          |          |
|             | Before surgery | 8  | 10.25         | 0.963    | −0.172   |
|             | After surgery | 11 | 21.27         | 0.0016   | −3.208   |

RT: Radiotherapy. *Compared to baseline.

### Table III. Tumour expression of transforming growth factor beta (TGFβ) before and after treatment compared to baseline.

| Group       | n  | TGFβ mean rank | p-Value | Z-value |
|-------------|----|----------------|---------|---------|
| 25 Gy RT    | 9  | 7.72           |         |         |
| Baseline    | 6  | 9.17           | 0.529   | −0.714  |
| Before surgery | 18 | 24.25         | <0.0001 | −4.010  |
| After surgery | 14 | 18.96         | <0.0001 | −3.862  |
| 50 Gy RT    | 10 | 6.95           |         |         |
| Baseline    | 2  | 8.00           | 1.000   | 0.000   |
| Before surgery | 14 | 18.96         | <0.0001 | −3.862  |
| After surgery | 11 | 18.73         | 0.0002  | −3.399  |
| No RT       | 8  | 8.12           |         |         |
| Baseline    | 5  | 5.80           | 0.435   | −0.897  |
| Before surgery | 11 | 18.73         | 0.0002  | −3.399  |
differences in the two RT regimes may support usage of the more favourable long course treatment, due to its limited effect on MMP7 expression, and consequently on reduced tumour progression. Members of our group have earlier shown the progressive expression of MMP7 from normal mucosa to cancer; MMP7 levels were up to 10-fold lower in normal mucosa compared to those in tumour samples (9, 17, 18).

A homogenous trend was observed in the expression of TGFB, as a marker of inflammation and fibrosis, in tumour before and after treatment compared to baseline. In the three groups, no significant increase of TGFB was observed after 25 and 50 Gy RT immediately before surgery. However, TGFB increased significantly, 2- to 3-fold only after surgery, with maximum increase in the 25 Gy irradiated group. TGFB participates in the remodelling of the ECM, but has many other functions, such as suppression of the immune system and regulation of cell growth. It was also shown to act both as an inhibitor of tumour growth and as a promoter of tumour progression (37, 38).

TGFB activated by radiation may be involved in the mechanisms of increased inflammation after RT eventually leading to fibrosis (39). A previous investigation from our group in fact showed that RT and surgery induced depression of TGFB in rats in the first postoperative week followed by up-regulation in the late period (38, 40). Similarly, other authors showed lower levels of active TGFB in both tumour tissue and rectal mucosa in patients irradiated for rectal cancer (38). Evidence points towards RT-induced activation of latent TGFB but this activation may only be seen in a limited time window, which may not have been captured in these studies.

In conclusion, we can state that surgery has an overriding effect on the up-regulation of MMP7 and the inflammatory cytokine TGFB. In our clinical setting, preoperative RT of 50 Gy induced significantly less MMP7 expression at surgery compared to short-term irradiation of 25 Gy. This difference supports usage of the more favourable long course treatment, for its limited effect on MMP7 expression and consequently on reducing tumour progression.

Although there is a wide body of literature available dealing mainly with short-term RT, to our knowledge there are no previous publications comparing the effects of short-and long-course RT on MMP7 expression in patients with rectal cancer. For this reason, the present study must be considered a pilot investigation on the modulation of expression by different RT courses in this setting. The main limitation of the present research was the low number of patients enrolled in the different groups. Further studies are needed to validate these findings and to better understand the pathways involved in progressive cancer disease through ECM remodelling under the influence of RT.

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