ORIGINAL ARTICLE

Rectal adenocarcinoma with oncocytic features: possible relationship with preoperative chemoradiotherapy

M Rouzbahman, S Serra, R Chetty

J Clin Pathol 2006;59:1039–1043. doi: 10.1136/jcp.2005.031997

See end of article for authors' affiliations

Correspondence to: R Chetty, Department of Pathology, University Health Network, Princess Margaret Hospital, 610 University Avenue, 4th Floor, Suite 302, Room 312, Toronto, ON M5G 2M9, Canada; runjan.chetty@uhn.on.ca

Accepted for publication 15 August 2005
Published Online First 7 February 2006

Background: The introduction of preoperative chemoradiation into the treatment protocol of rectal adenocarcinomas has affected the microscopical morphology in subsequent resection specimens. The constellation of histopathological changes is varied and well documented.

Aim: To describe oncocytic change in rectal cancers that have been treated with chemoradiation before surgery.

Methods: 7 of 54 patients with rectal cancer were identified with a history of chemoradiation, specifically directed to the rectal tumours in fractions of 4500–5000 cGy of radiation and 5-fluorouracil. The rectal tumours in five of these seven patients were composed of oncocytes that constituted 30–80% of the cancers. The patients were three men and two women aged 65–73 years, all with T3 N0 tumours. The intervals between chemoradiation and resection varied from 3 to 12 weeks.

Results: The tumour cells conformed to oncocytes morphologically (large size with abundant, granular eosinophilic cytoplasm, vesicular nuclei and prominent acidophilic nucleoli), immunohistochemically (positive for carcinoembryonic antigen, cytokeratin 20 and caudal type homeo box transcription factor 2, but negative for both chromogranin and synaptophysin) and ultrastructurally (large cells showing tight junctions, cytoplasmic engorgement by mitochondria and absence of neurosecretory granules).

Conclusions: The changes in these cells differ from those described previously in endocrine cells encountered in pretreated rectal cancers. 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. Oncocytic change may be an under-recognised histopathological change in rectal cancers receiving preoperative chemoradiation.

Neoplasms that are composed of large cells with abundant granular, eosinophilic cytoplasm represent a distinctive type of tumour, dubbed “oncocytic”, and are encountered in several organs. An abundance of mitochondria in the cytoplasm is the hallmark of these cells, and imparts the oncocytic or deeply eosinophilic, granular appearance to the cells in haematoxylin and eosin-stained sections. This tinctorial characteristic—the large cell size, a vesicular nucleus and a prominent acidophilic nucleolus—constitute the oncocytic phenotype. However, it is noteworthy that an eosinophilic cytoplasm can also be due to other non-ribosomal intracellular organelles, such as neurosecretory granules, lysosomes, secretory (exocrine) granules, cytoplasmic filaments and smooth endoplasmic reticulum.¹ Such cells, however, lack the abundant cytoplasm and nuclear features associated with oncocytes. Oncocytic and oxyphilic cells are synonymous and are due to intracytoplasmic mitochondria.

Tumours containing oncocytes occur more often in some sites, whereas they represent rare histological variants in others. Oncocytic tumours form a distinctive subtype of tumour in the thyroid gland (Hürthle cell tumours),² kidney (oncocytoma),³ salivary glands (as oncocytosis, oncocytic nodules or as part of a Warthin's tumour)⁴ and in endocrine glands such as the pituitary, parathyroid and adrenal glands (in adenomas and carcinomas).^{4–6} Tumours containing oncocytes can also occur rarely in other sites such as the ovary,^{7–8} lung,^{6–9–10} breast,¹¹ skin¹² and soft tissue.¹³

Although several tumours with prominent “pink” (eosinophilic) cytoplasm are recognisable in the digestive tract, tumours with oncocytic features rarely affect the

gastrointestinal tract, liver and pancreas.¹⁴ Adenocarcinomas with oncocytic features have also been encountered in the stomach^{15–17} and in the small intestine.⁶ Two recent publications mention the presence of eosinophilic cells in rectal adenocarcinomas after the patients underwent preoperative chemoradiotherapy.^{18–19} However, these cells were thought to be endocrine cells and no mention is made of true oncocytic change in this clinical context.

We describe the clinicopathological, immunohistochemical and ultrastructural findings of a series of five rectal adenocarcinomas showing oncocytic features after the patients received preoperative chemoradiation.

MATERIALS AND METHODS

The pathology department computer records at University Health Network, Toronto, Canada, were searched for resection specimens of rectal adenocarcinomas between 2001 and 2005. The initial diagnostic (pretreatment) biopsy specimens, where available, of patients who received preresection chemoradiation were also sought and retrieved. All haematoxylin and eosin-stained slides of the patients were reviewed, with the investigators looking specifically for oncocytes. These cells were defined as large cells with abundant eosinophilic granular cytoplasm, containing large vesicular nuclei and prominent acidophilic nucleoli. For the purposes of this study, only patients whose glands were composed entirely of oncocytic cells were included; those with occasional single oncocytes were excluded. The resection specimens were fixed in 10% neutral buffered formalin, grossed as per the standard departmental protocol and processed routinely.

Biopsy samples with oncocytic features were stained with phosphotungstic acid haematoxylin, carcinoembryonic antigen, cytokeratin 7, CK20, caudal type homeo box transcription factor 2, synaptophysin and chromogranin. Immunohistochemical analysis was carried out using the standard streptavidin–biotin complex technique, after microwave antigen retrieval on 4-µm paraffin-wax sections of formalin-fixed, paraffin-wax-embedded tissue. Appropriate positive and negative controls were run in parallel.

For ultrastructural analysis, tissue for electron microscopy was taken from the formalin-wax-fixed gross specimen in one patient and from the paraffin-wax-embedded block in another patient. The specimens were fixed in 2% glutaraldehyde and were processed routinely.

RESULTS

A total of 54 cases of rectal adenocarcinoma were retrieved from the files. Of these, seven patients had a history of chemoradiation before resection of their rectal tumours. Five of the seven samples contained an oncocytic component, which constituted 30–80% of the tumour, thus warranting the appellation adenocarcinoma with oncocytic change. In four of these samples with an oncocytic component, the pretreatment diagnostic biopsy samples were re-evaluated and none showed oncocytes. The samples of the remaining two patients who underwent preoperative chemoradiation contained isolated, occasional oncocytic cells in malignant glands, which otherwise appeared as conventional adenocarcinomas.

Of the 47 patients who did not receive preoperative chemoradiation for rectal adenocarcinoma, three received postoperative adjuvant chemoradiotherapy and subsequently developed recurrent cancers, which were also reviewed. None of these three patients had an appreciable oncocytic population of tumour cells other than the desultory oncocyte singly interspersed among the usual malignant columnar cells—in other words, glandular structures composed completely of oncocytes were not observed.

Table 1 summarises the clinicopathological features of the patients.

The patients ranged in age from 65 to 73 years and all had T3 N0 rectal adenocarcinomas. All patients received the same preoperative chemoradiotherapy regimen of 4500–5000 cGy of radiation in 25 fractions and 5-fluorouracil infusion with leucovorin. The period between chemoradiation and tumour resection varied from 3 to 12 weeks.

Follow-up

Patients 1 and 2 are alive and well 4 months postoperatively. Patient 3 presented 18 months after resection with lung metastases. Patient 4 was lost to follow-up after 6 months

and patient 5 died of abdominal sepsis in a week after the operation.

Microscopy

The resection specimens from patients who did not undergo preoperative chemoradiation did not contain any oncocytes, not even focally.

All the five samples of adenocarcinoma with oncocytic features showed similar microscopical features. Large areas of coagulative necrosis, granulation tissue, fibrosis and focal areas of acellular extravasated mucin were present. Surviving adenocarcinoma was composed of glands containing oncocytic cells, with large, eosinophilic granular cytoplasm, large nuclei and prominent acidophilic nucleoli (fig 1). These cells also manifested marked pleomorphism and occasional mitotic mitoses; some showed cytoplasmic vacuolation. Typical or usual adenocarcinoma was present, somewhat sharply demarcated from the oncocytic component (fig 2). Interestingly, the typical areas tended to be superficial, whereas the oncocytic foci were located deeper and at the infiltrating front of the tumours.

The phosphotungstic acid haematoxylin stain highlighted the cytoplasmic granularity of the tumours. Cells morphologically consistent with endocrine cells were noted in the components of conventional adenocarcinoma.

Immunohistochemistry

The tumours, including the oncocytic cells, were positive for cytokeratin 20, carcinoembryonic antigen caudal type homeo

Figure 1 Residual rectal adenocarcinoma (sample 1) after preoperative chemoradiation, showing the tumour to be composed of cells with abundant eosinophilic cytoplasm. The cells morphologically conformed to the definition of oncocytes.

Patient no.	Age	Sex	Clinical presentation	Stage	Grade	Oncocytes (%)	Interval between chemoradiotherapy and surgery (weeks)
1	65	M	Rectal bleeding	T3 N0	Moderately differentiated	80	8
2	71	F	Abdominal pain, bloody mucus discharge	T3 N0	Moderately differentiated	50	12
3	54	M	Rectal bleeding	T3 N0	Moderately differentiated	50	4
4	68	M	Rectal bleeding	T3 N0	Poorly differentiated	30	4
5	73	F	Rectal bleeding	T3 N0	Moderately differentiated	80	3

F, female; M, male.

Figure 2 Abrupt transition between the conventional adenocarcinoma and areas composed of oncocytic glands. The conventional adenocarcinoma tended to occupy the more superficial areas of the tumour, whereas the oncocytic glands were located deeper and at the invasive front of the cancer. The granular, eosinophilic cytoplasm and vesicular nuclei with prominent, acidophilic large nucleoli can be seen.

box and transcription factor 2, and negative for cytokeratin 7. In particular, the oncocytic cells were completely negative for both chromogranin and synaptophysin. Occasional chromogranin and synaptophysin cells were present in the non-oncocytic component, corresponding to the cells thought to be endocrine cells in the haematoxylin and eosin-stained sections (fig 3A). Endocrine cells in normal mucosa served as an internal positive control. The oncocytic cells were negative for chromogranin (fig 3B).

Electron microscopy

Ultrastructural analysis showed the cytoplasm of oncocytic tumour cells to be packed with numerous mitochondria, confirming the results of haematoxylin and eosin staining (fig 4A, B). Desmosomes linking adjacent cells were noted. Despite an assiduous search, we did not observe any neurosecretory granules in both cases.

DISCUSSION

Several histological variants of rectal adenocarcinoma, some with prognostic relevance, such as signet ring, mucinous, medullary, adenosquamous (including glassy cell), undifferentiated, spindle cell, clear cell and hepatoid,²⁰ have been recognised. To our knowledge, there has been only one report of an oncocytic adenocarcinoma of the rectum documented so far.²¹

In 1931, Hamperl²² used the term “oncocyte” (Greek—swollen cell) for the first time to describe large eosinophilic polyhedral cells similar to those that had been reported in canine thyroid by Hürthle.²³

An oncocyte obtains its characteristic cytoplasmic appearance from the presence of numerous normal or variant-form mitochondria.²⁴ Although it is possible to recognise an oncocyte on conventional haematoxylin and eosin-stained slides, enzymatic reactions for endogenous biotin, immunohistochemical analysis using antimitochondrial antibodies and electron microscopy are adjunctive studies that can be used to show the presence of mitochondria in large numbers. The exact aetiology of this ultrastructural change resulting in the oncocytic morphology is unknown. It could be a degenerative process, a structural defect, a cell response to hypoxia or other forms of cell injury, or possibly even the result of a genetic abnormality. Whether mitochondrial accumulation has functional implications is not clear, although oncocytic cells are thought to be relatively inert.¹⁴

Oncocytic change has varying clinical relevance, largely dependent on the site of occurrence. Hürthle cell tumours of thyroid, oncocytoma of kidney and fibrolamellar carcinoma of liver are examples of tumours composed of oncocytes but with different clinical relevance that is site-related. Hürthle cell change can be found in a variety of benign and malignant conditions in the thyroid and is not of prognostic relevance by itself. On the other hand, fibrolamellar carcinoma has a better prognosis than conventional (non-oncocytic) hepatocellular carcinoma,¹⁴ and renal oncocytomas are accompanied by indolent, benign behaviour.

Oncocytic adenocarcinoma of the stomach is an uncommon variant of gastric cancer that occurs particularly in older patients.^{15–16} Although it has been suggested that it has a better prognosis than usual gastric adenocarcinoma, few cases have been encountered and followed up to be categorical.¹⁶ The oncocytic adenocarcinoma of the rectum reported by Piscitelli *et al*²¹ was composed wholly of oncocytic cells without any conventional rectal cancer foci. Furthermore, this patient did not undergo preoperative chemoradiation. An interesting histological variation was the presence of numerous intraluminal, basophilic and non-birefringent microcalcifications.²¹ This finding was not encountered in the five patients in this series. A relatively recent addition to rectal cancer treatment protocols is preoperative radiation with or without chemotherapy. Evidence suggests that neoadjuvant treatment downstages the disease, improves local control, occasionally eradicates and ultimately boosts survival in patients with rectal cancer.^{18–25} This has obviously affected the morphology of the tumours, and specific and characteristic changes have been documented.^{19–25} These changes were tabulated by Wheeler *et al*²⁵ and include tumour necrosis, stromal fibrosis,

Figure 3 Chromogranin staining of conventional adenocarcinoma (A) with occasional positive endocrine cells; (B) the oncocytic component negative for chromogranin.

Figure 4 (A, B) Ultrastructural examination of the oncocytic cells (specimen 2) confirmed their epithelial nature (presence of desmosomes) and showed the characteristic cytoplasmic distension by myriads of mitochondria. Neurosecretory granules were not seen.

changes in the advancing tumour edge, foreign body giant cells at the site of tumour, absence of tumour, with replacement by fibro-inflammatory tissue, absence of vascular invasion, decreased lymphocytic response and colitis cystica profunda. Another study described increased mucin production with the formation of stromal mucin pools; marked cytoplasmic eosinophilia, often in combination with marked nuclear atypia but without active mitoses in tumour cells showing treatment effect, an endocrine tumour phenotype in most of the eosinophilic appearing cells; and retention of a mucosal adenoma (if present) and dysplastic change in the presence of tumour regression in the bowel wall.¹⁸ In this study, eosinophilic change was the commonest change encountered in adenocarcinomas after chemoradiation, with about 30% of samples containing >50% of tumour cells displaying “marked cytoplasmic eosinophilia”.¹⁸ The authors ascribed the cytoplasmic eosinophilia as evidence of endocrine cell change, the morphology being corroborated by positive neuroendocrine immunohistochemical markers.^{18, 19}

The morphological description rendered by Shia *et al*¹⁹ describes some cells with abundant eosinophilic cytoplasm, vesicular nuclei and large eosinophilic nucleoli. This morphology is also compatible with the description of oncocytes. It should be remembered that none of the oncocytic cells in the five patients described in this study showed any immunoreactivity with synaptophysin and chromogranin. In addition, electron microscopical examination of two specimens (which were morphologically and immunophenotypically identical to the other three specimens) showed the cytoplasm to be engorged with mitochondria. Furthermore, neurosecretory granules were not seen. Thus, there is clear evidence that the large cells with abundant granular, eosinophilic cytoplasm, vesicular nuclei and prominent acidophilic nucleoli in our five specimens are oncocytes rather than endocrine cells. The cytomorphology of the oncocytes in this study is perfectly consistent with the cells in the case described as an oncocytic adenocarcinoma of the rectum by Piscitelli *et al*.²¹ This similarity is further strengthened by the identical immunoprofiles and ultrastructural findings.

It is plausible that not all deeply eosinophilic cells encountered in post-treated rectal adenocarcinomas are of endocrine cell lineage. We concur entirely with Shia *et al*^{18, 19} that endocrine cells are encountered in these rectal cancers, more so than in cases without preoperative treatment. However, we submit that some eosinophilic cells in pre-treated rectal cancers are oncocytes. The exact mechanism and pathogenesis of the oncocytic change is somewhat conjectural, but hypoxia with degeneration and subsequent structural aberration triggered by chemoradiation is the most likely sequence of events.

The obvious conundrum is to explain the occurrence of the oncocytic rectal adenocarcinoma as described by Piscitelli *et al*,²¹ without antecedent chemoradiation, and to deal with the allied question of why oncocytic change occurs in only some of the preoperatively treated cases. Oncocytic cells may be present in most, if not all, cases, at least focally. The number and extent of oncocytic cell change may be related to host factors (perhaps even an idiosyncratic response) and as yet unidentified tumour characteristics. In cases where no preceding chemoradiation was given, the oncocytic change may be due to a sporadic, random degenerative phenomenon, as seen in oncocytic lesions in other sites. In view of the small number of cases and limited follow-up periods, it would not be prudent to speculate on the biological significance or prognostic implications of oncocytes in pretreated rectal adenocarcinomas. However, oncocytic change is thought to be a consequence of chemoradiation and hence should be considered another histological stigma of treatment-induced change in rectal adenocarcinomas.

The purpose of this paper is therefore to highlight oncocytic change in rectal cancers that have been treated with preoperative chemoradiation. While cells with abundant eosinophilic cytoplasm have been documented in carcinomas post-chemoradiation, oncocytic change (ie, cytoplasm packed with mitochondria) has not been documented previously in this context. These cells are different from the deeply eosinophilic cells described by others, as they are oncocytes rather than endocrine cells, as shown morphologically, immunohistochemically and ultrastructurally.

Authors' affiliations

M Rouzbahman, S Serra, R Chetty, Department of Pathology, University Health Network; Toronto Medical Laboratories, University of Toronto, Toronto, Canada

Competing interests: None declared.

REFERENCES

- 1 Nappi O, Ferrara G, Wick MR. Neoplasms composed of eosinophilic polygonal cells: an overview with consideration of different cytomorphologic patterns. *Semin Diagn Pathol* 1999;**16**:82–90.
- 2 Rosai J, Carcangiu ML, DeLellis RA. In: *Tumors of the thyroid, Atlas of tumor pathology*. 3rd series, fascicle 5. Washington DC: Armed Forces Institute of Pathology 1992:161–82.
- 3 Perez-Ordóñez B, Hamed G, Campbell S, *et al*. Renal oncocytoma: a clinicopathologic study of 70 cases. *Am J Surg Pathol* 1997;**21**:871–83.
- 4 Giovanni T. Oncocytic tumours. *Virchows Arch* 1998;**433**:5–12.
- 5 Bisceglia M, Ludovico O, Di Mattia A, *et al*. Adrenocortical oncocytic tumors: report of 10 cases and review of the literature. *Int J Surg Pathol* 2004;**12**:231–43.
- 6 Smirnova EA, Mikhailov IG. Electron microscopic characteristics of oncocytoma of the lung, small intestine and adrenal gland. *Ark Patol* 1986;**48**:79–81.

- 7 **Miranda D**, Leite VH, de Azevedo GM Jr. Oncocytic adenocarcinoma of the ovary. *Virchows Arch* 1996;**429**:181–3.
- 8 **Pitman MB**, Young RH, Clement PB, *et al.* Endometrioid carcinoma of the ovary and endometrium, oxyphilic cell type: a report of nine cases. *Int J Gynecol Pathol* 1994;**13**:290–301.
- 9 **Fernandez MA**, Nyssen J. Oncocytoma of the lung. *Can J Surg* 1982;**25**:332–3.
- 10 **Santos-Briz A**, Terron J, Sastre R, *et al.* Oncocytoma of the lung. *Cancer* 1977;**40**:1330–6.
- 11 **Damiani S**, Eusebi V, Losi L, *et al.* Oncocytic carcinoma (malignant oncocytoma) of the breast. *Am J Surg Pathol* 1998;**22**:221–30.
- 12 **Roth MJ**, Stern JB, Hijazi Y, *et al.* Oncocytic nodular hidradenoma. *Am J Dermatopathol* 1996;**18**:314–6.
- 13 **Polk P**, Parker KM, Biggs PJ. Soft tissue oncocytoma. *Hum Pathol* 1996;**27**:206–8.
- 14 **Papotti M**, Cassoni P, Taraglio S, *et al.* Oncocytic and oncocytoid tumors of the exocrine pancreas, liver, and gastrointestinal tract. *Semin Diagn Pathol* 1999;**16**:126–34.
- 15 **Caruso RA**, Fabiano V, Rigoli L, *et al.* Focal parietal cell differentiation in a well-differentiated (intestinal-type) early gastric cancer. *Ultrastruct Pathol* 2000;**24**:417–22.
- 16 **Takubo K**, Honma N, Sawabe M, *et al.* Oncocytic adenocarcinoma of the stomach: parietal cell carcinoma. *Am J Surg Pathol* 2002;**26**:458–65.
- 17 **Yang GY**, Liao J, Cassai ND, *et al.* Parietal cell carcinoma of gastric cardia: immunophenotype and ultrastructure. *Ultrastruct Pathol* 2003;**27**:87–94.
- 18 **Shia J**, Guillem JG, Moore HG, *et al.* Patterns of morphologic alteration in residual rectal carcinoma following preoperative chemoradiation and their association with long-term outcome. *Am J Surg Pathol* 2004;**28**:215–23.
- 19 **Shia J**, Tickoo SK, Guillem JG, *et al.* Increased endocrine cells in treated rectal adenocarcinomas: a possible reflection of endocrine differentiation in tumor cells induced by chemotherapy and radiotherapy. *Am J Surg Pathol* 2002;**26**:863–72.
- 20 **Hamilton SR**, Vogelstein B, Kudo S, *et al.* Pathology and genetics of the tumours of digestive system. In: Hamilton SR, Aaltonen LA, eds. *WHO classification of tumours*. Lyon: International Agency for Research on Cancer, 2000:103–43.
- 21 **Piscitelli D**, Ingravalle G, Resta L, *et al.* Oncocytic adenocarcinoma of the rectum with diffuse intra-luminal microcalcifications: the first reported case. *Virchows Arch* 2003;**443**:579–82.
- 22 **Homperl H**. Beiträge zur normalen und pathologischen Histologie menschlicher speicheldrüsen. *Z Mikr Forschung* 1931;**27**:1–11.
- 23 **Hurthle K**. Beiträge zur kenntnis der sekretion svarganges in der Scilddrüse. *Arch F D Ges Physiol* 1894;**56**:1–7.
- 24 **Chang A**, Harawi SJ. Oncocytes, oncocytosis, and oncocytic tumors. *Pathol Annu* 1992;**27**:263–304.
- 25 **Wheeler JM**, Warren BF, Jones AC, *et al.* Preoperative radiotherapy for rectal cancer: implications for surgeons, pathologists and radiologists. *Br J Surg* 1999;**86**:1108–20.