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Sphincter preservation in rectal cancer after radiotherapy

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Purpose: To present clinical data supporting the hypothesis that radiotherapy (RXT) can play a role in the sphincter saving treatment of rectal cancer.

Methods: RXT can provide sphincter saving approach in 2 situations. (1) Radiotherapy alone in T1 N0 (contact x-ray) or T2-3 N0-1 lesions in inoperable patient (combined contact x-ray + External RXT + iridium Brachytherapy). (2) Preoperative RXT with long interval (5 weeks) before surgery in T2-3 lesions. Randomized trial comparing immediate v.s delayed surgery.

Results: 1) T1 N0 contact x-ray: 116 patients treated between 1980–1998: local control 89% overall 5 year survival rate 83%. T2-3 combined irradiation: 1986–1997, 63 patients: local control: 71% overall 5 year survival: 64% (Gérard Sem. Rad. Oncol. 1998, 8: 13).

2) Lyon R90.01 phase III trials. Between 1990–95, 203 patients included short interval () long interval (). The long interval showed a significant increase in complete sterilization of the operative specimen (15% vs 5%) and a trend toward more sphincter saving surgery for low lying tumor (41% vs 22%). Updata analysis after 5 year follow up showw no difference in term of local control and 5 year survival (François J.C.O 1999, 17: 2396).

Conclusion: 1) High dose of RXT in small volume can give long term control of T1-2 (3) rectal cancer. 2) Preoperative RXT and delayed surgery may increase the chance of sphincter saving surgery.

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How mandatory is preoperative short term radiotherapy in addition to TME-surgery for rectal cancer

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Both short term preoperative radiotherapy and total circumferential mesorectal excision have independently demonstrated to improve local control and rectal cancer. The combination of the two treatment modalities without radiotherapy has never been evaluated in the structure of a multicenter randomized clinical trial. The Dutch Colorectal Cancer Group jointly with the Nordic Gastro Intestinal Tumor Adjuvant Therapy and the EORTC conducted a randomized trial to answer the question whether preoperative short term radiotherapy is beneficial in TME treated patients.

Methods: Between January 1996 and January 2000 1861 patients with resectable rectal cancer were randomly assigned to preoperative 5 × 5 GY radiotherapy followed by TME of TME alone. In the trial standardization and quality of surgery, pathology and radiotherapy was achieved, where-as no postoperative chemotherapy was given due to the result of an earlier clinical trial of this group in which no beneficial effect of chemotherapy was found, however in non-standardized surgery.

Results: Of 1861 patients, 57 patients were ineligible and excluded from analysis. Overall two-year local recurrence rate was 5.8% after local radical resection. The two-year local recurrence rates were 8.5% in the TME alone group and 2.9% in the preoperative radiotherapy plus TME-group. Preoperative radiotherapy reduced local recurrence rate for tumors located below 10 centimeter of the anal verge. A significant effect of radiotherapy was found only in TNM stages II and III. The survival in both randomized groups was similar. Quality assurance led to a major improvement of quality of life through reduction of abdominal perineal resections and nerve preservation. Quality assurance of pathology could identify poor surgical specimens with a direct relationship with recurrence and survival, but overall short-term preoperative radiotherapy at this time of analysis has an effect on local recurrence without an effect on survival. Detailed analysis of all subgroups including quality of life will be presented. The current study of the Dutch Colorectal Cancer Group (PROCTOR-study) evaluates after preoperative radiotherapy and TME-surgery the value of postoperative chemotherapy in stage II and III rectal cancer.

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Regulation of p53 activity: the cancer connection

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The p53 tumor suppressor gene is a major target for inactivation in human cancer. In colorectal cancer (CRC), over 80% of the tumors carry mutations in the p53 gene, which are believed to occur late during tumor progression. Aberrant activation of beta catenin, either by inactivation of the APC tumor suppressor or by mutation of the beta catenin gene itself, occurs very early in colorectal carcinogenesis and plays an important role in tumor initiation.

We investigated whether there is a connection between the activation of beta catenin and the inactivation of p53. We report that deregulated beta catenin leads to accumulation of stable, transcriptionally active p53. This is due to inhibition of Mdm2-mediated degradation of p53, which is responsible for maintaining low levels of p53 in normal cells. Induction of p53 by beta catenin is mediated by the ARF tumor suppressor, and ARF is essential for this induction. Deregulated beta catenin stimulates the transcriptional activity of the ARF promoter. In primary fibroblasts the induction of p53 by excess beta catenin elicits a senescence-like growth arrest; this does not occur in cells lacking either p53 or ARF. It is proposed that these observations underscore an anti-tumor mechanism where deregulation of beta catenin evokes a p53-response, which arrests cell proliferation and prohibits further tumor progression. When p53 function is lost, the oncogenic effects of deregulated beta catenin are unleashed and are enabled to drive tumor progression.

Importantly, cells derived from CRC exhibit constitutive activity of the ARF promoter, which can be blocked by inactivation of beta catenin-mediated signaling. Furthermore, recent work from several groups indicates that ARF transcription is abrogated in a substantial fraction of CRC cases by promoter methylation. This silencing, occurring early during tumorigenesis, is predicted to abrogate the induction of p53 by deregulated beta catenin, thereby overcoming a major obstacle to tumor progression

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The p53 family: What implications in human carcinogenesis?

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Many tumors overexpress mutant forms of p53. A growing number of studies suggest that the nature of a p53 mutation in a cell can impact upon cellular properties, clinical responses to therapy and prognosis of a tumor. We and others have previously reported that conformational p53 mutants such as p53His175, but not DNA contact mutants, can increase the resistance to etoposide or contribute to genomic instability by abrogating the mitotic spindle checkpoint and consequently leading to polyploidy of human cells. The molecular mechanisms underlying such effects of mutant p53 remain to be elucidated. We may depict the two following scenarios: A) mutant p53 can transactivate or repress target genes through the binding to specific DNA consensus different from those bound by wt-p53 or through the physical association with DNA binding proteins and utilization of the p53 transactivation domain to turn on/off specific genes; B) mutant p53 can associate with and sequester proteins that are required for anti-tumor effects such as growth inhibition and apoptosis. The recent identification of two p53 relatives, such as p73 and p63 holds new perspectives in studying gain of function of mutant p53. p73 and p63 share a significant homology each other and with p53. Several p73 and p63 isoforms are present in cells. They result either from the use of a cryptic promoter or by alternative splicing. Ectopic expression of p73 or p63 in p53 +/+ or p53 −/− cancer cells can recapitulate the most characterized p53 biological activities such as growth inhibition, apoptosis, and differentiation. The existence of heterodimers between mutant p53 and p73 or p63 has recently been reported. We have previously shown that mutant p53 binds in vitro and in vivo to different isoforms of p73 and markedly reduce their transcriptional activity. Here we report that human tumor-derived p53 mutants can associate in vitro and in vivo with p63. This association is mediated by the core domain of mutant p53 and the DNA binding domain of p63 respectively and may occur directly. We show that overexpression of mutant p53 impairs *in vitro* and *in vivo* sequence specific DNA binding of p63 and consequently affects

its transcriptional activity. Furthermore, p63-induced growth inhibition is markedly counteracted by mutant p53. Thus, inactivation of p53 family members may contribute to the biological properties of p53 mutants in promoting tumorigenesis and in conferring selective survival advantage to cancer cells.

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p53 diagnosis in human cancer: what utility?

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The tumour suppressor p53 is a phosphoprotein barely detectable in the nucleus of normal cells. Upon cellular stress, particularly that induced by DNA damage, p53 can arrest cell cycle progression thus allowing the DNA to be repaired or it can lead to apoptosis. In cancer cells bearing a mutant p53, this protein is no longer able to control cell proliferation, resulting in inefficient DNA repair and the emergence of genetically unstable cells. Three approaches can be used to test p53 alteration in human tumour: molecular, immunocytochemical and serological diagnosis. i) Molecular analysis DNA sequencing led to the determination of the exact mutational event that modified the p53 gene. ii) Immunocytochemical analysis p53 mutations induce a change in the conformation of the p53 protein leading to the stabilisation and the accumulation of p53 in the nucleus of tumoural cell. This observation has encouraged an intensive study of the expression of p53 protein by immunohistochemistry in a large panel of tumours, since there seems to be a good correlation between p53 gene mutation and protein accumulation iii) Serological analysis We demonstrate that p53-Abs are found predominantly in human cancer patients with a specificity of 96%. Such antibodies are predominantly associated with p53 gene missense mutations and p53 accumulation in the tumour. It has been demonstrated that this immune response is due to a self-immunisation process linked to the strong immunogenicity of the p53 protein.

The detection of p53 mutation in human tumours have been extensively studied for molecular epidemiology or for clinical evaluation in order to link p53 deficiency to therapy failure or prognosis. Due to an important heterogeneity in the methods used for the detection of p53 alteration, there is a huge controversy concerning the clinical value of p53 alteration. These points will be discussed in the view of recent information concerning p53: i) the inadequate strategy used by the majority of laboratories to analyse p53 status; ii) strong evidence in favour of the heterogeneous behaviour of various mutant p53 proteins; iii) discovery of p53 family members, p63/p73 and iv) association of codon 72 polymorphism (Arg/Pro) and specific properties of the protein. We will conclude with some recommendations concerning the strategy to be used for an unbiased analysis of p53 alterations in human tumours and its potential benefit in clinical practice.

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Predictive value of p53 alterations to therapy in cancer; critical evaluation of clinical and laboratory methods

Abstract not received.

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p53-induced apoptosis and new cancer therapy

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The p53 tumor suppressor protein inhibits tumor growth largely through its ability to induce apoptosis. p53 induces apoptosis through transactivation and transrepression of specific target genes. Around 50% of all human tumors carry p53 point mutations that disrupt p53:s specific DNA binding and thus p53-mediated target gene regulation.

Since p53 mutation is frequent in human tumors of various types, new therapeutic strategies based on restoration of wild type p53 function should be applicable to a large number of tumors. One such approach is pharmacological reactivation of mutant p53 proteins. Mutant p53 is often expressed at high levels in tumor cells and so mutant p53 reactivation should trigger massive apoptosis and eliminate the tumor. In order to identify low molecular compounds that can restore wild type function to mutant p53, we screened a chemical library from the National Cancer Institute. We identified one compound that was capable of inducing apoptosis in human tumor cells in a manner dependent on mutant p53 expression. This compound could restore the specific DNA binding and preserve the active conformation of mutant p53 proteins in vitro. Moreover, it restored both wild type p53 conformation and the transactivation function of mutant p53 in living cells. The molecule was able to reactivate a wide variety of tumor-derived mutant p53 proteins. In vivo experiments demonstrated inhibition of xenograft tumor growth with no apparent toxicity. This compound represents a first step towards the development of anticancer drugs that specifically target mutant p53.

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Improvement in tumour staging

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Overall prognosis of patients with oesophageal carcinoma remains poor, mainly due to the late presentation and advanced stage of the disease at the time of presentation.

The therapeutic option in oesophageal cancer management depends on the clinical staging and includes radical surgery in early disease, multimodal treatment schemes combining neo-adjuvant chemoradiotherapy followed by surgery in locally advanced disease and palliative treatment in cases of distant metastatic disease.

Over the last decades, clinical staging has improved since the introduction of CT-scan and transoesophageal endoscopic ultrasound (EUS). However, performing threefield lymphadenectomy we found that 20% of the patients with T3N1 adenocarcinoma of the distal oesophagus had unexpectedly positive lymphnodes in the cervical region.

As for the T-factor, EUS is especially valuable in assessing transmural extent of the tumour provided the tumour can be passed. The T-factor is often overestimated on CT-scan.

As for the local nodes (N1), CT-scan and EUS have a high sensitivity but a low specificity. Newer techniques, such as EUS guided biopsy aim to overcome the difficulties in differentiation between benign and malignant nodes.

Some centers propagate the routine use of minimally invasive surgical staging procedures such as thoracoscopy and laparoscopy in the evaluation of nodal involvement and allowing the detection of unimaged pleural or peritoneal disease. These techniques however are time consuming and have their own morbidity.

We evaluated the use of PET-scan in preoperative staging of oesophageal carcinoma (n = 74). The sensitivity and specificity for detection of stage 4 disease was statistically significantly better than for CT + EUS. The additional value in detecting stage 4 disease with PET-scan was 16%.

PET scan showed to have a low sensitivity (22%) in detecting locregional lymphnodes. For detection of metastatic lymphnode involvement however, the sensitivity of PET scan was 77% which was higher than the sensitivity of CT + EUS (46%).

PET scan has a high specificity, both for local lymphnode as for metastatic lymphnode involvement (90%).

We conclude that clinical staging for oesophageal cancer remains poor, probably because of the extensive and unpredictable lymphatic spread. PET-scan however has an important role in this staging. PET scan also seems to have an important role in assessing tumour response in induction treatment schemes.

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Surgery: extent of resection

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Overall prognosis in patients with carcinoma of the esophagus and GEJ remains poor mainly due to the late presentation and advanced stage at the time of diagnosis. Over the last decades postoperative mortality has substantially decreased from approximately 15% before the eighties to approximately 5% in high volume centers.

A complete resection (R0) is the goal of any surgery for cure. Much debate however persists on extent of surgery.

The options are standard resection, usually transhiatal versus extended resection and lymphadenectomy, mostly performed transthoracically. More radical surgery definitely results in better staging and prolonged tumour free survival. Although proof is lacking data from literature suggests higher cure rates after more extensive surgery with five year survival rates of approximately 35% after R0 resection, whereas reported 5-year survival rates after standard resection mostly are below 25%.