

## **ROLE OF APOPTOSIS AND CELL CYCLE CONTROL FOR RESPONSE AND SURVIVAL OF COLORECTAL CANCER PATIENTS TREATED WITH PREOPERATIVE RADIOCHEMOTHERAPY ± HYPERTHERMIA**

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### **Background**

Cancer develops in consequence of both disrupted cell cycle control and apoptosis. It is well known that cytotoxic drugs, ionizing gamma-irradiation (IR) and heat shock (hyperthermia) induce apoptosis in tumor cells. Nevertheless, the cellular pathway components involved in therapy resistance and associated with local tumor control and patient survival are still under investigation. We analysed parts of the p53-signalling pathway in tumor samples from a study in 66 patients with rectal carcinoma treated by a neoadjuvant approach with radiochemotherapy with or without hyperthermia.

### **Methods**

Expression of p53, BAX, p21<sup>WAF1/CIP1</sup>, and Ki-67 was investigated by immunohistochemistry in pre- and post-therapeutic tumor samples in 66 patients. Tumor DNA was screened for p53 mutations by SSCP-PCR. Paired tumor samples (pre-therapy and post-therapy) were collected prospectively. The functional relevance of p53, p21<sup>CIP/WAF-1</sup> and Bax was investigated in isogenic HCT116 cell mutants treated with 5-fluorouracil (5-FU), IR and heat shock.

### **Results**

Local tumor response was linked to expression of p21<sup>CIP/WAF-1</sup> ( $p < 0.05$ ), but not p53 expression or mutation. Rectal carcinoma patients who received an optimal heat shock treatment showed a response that correlated well with Bax expression ( $p = 0.018$ ). This dichotomy of p53 pathway components regulating response to therapy was confirmed in vitro. In isogenic HCT116 cell mutants, loss of Bax but not p53 or p21<sup>CIP/WAF-1</sup> resulted in resistance against heat shock. In contrast, loss of p21<sup>CIP/WAF-1</sup> or, to a lesser extent, p53 sensitized predominantly for 5-FU and IR.

Concerning the overall survival, patients with a decrease in p21<sup>WAF1/CIP1</sup> expression following radiochemotherapy had better disease-free survival ( $p = 0.03$ ). Similarly, patients with an increase in proliferative activity as measured by increased Ki-67 expression post-therapy had better disease-free survival ( $p < 0.005$ ). In contrast, pretherapeutic levels of p53, BAX or p21 expression and p53 mutation had no prognostic value, indicating that the combination of radiotherapy and chemotherapy might override defects in these genes.

### **Conclusion**

Our data support the clinical relevance of p21 in the suppression of both, proliferation and apoptosis. Induction of p21 represents a novel resistance mechanism in rectal cancer undergoing preoperative radiochemotherapy. The clinical data together with the in vitro data establish a different impact of p53 pathway components on treatment responses. While chemotherapy and IR depend primarily on cell cycle control (p21), heat shock depends primarily on Bax

while p53 status poorly correlates with response. These analyses therefore provide a rational approach for dissecting the mode of action of treatment modalities that may be employed to circumvent clinically relevant resistance mechanisms in rectal cancer.