Nature Reviews Clinical Oncology **8**, 508 (2011); published online 26 July 2011; doi:10.1038/nrclinonc.2011.112

RADIOTHERAPY

Repopulating tumor cells—dying for caspase 3

During chemotherapy or radiotherapy treatment cells die as a result of the damage caused by such cytotoxic therapy. It has been assumed that dying or dead cells are absorbed by macrophages or other surviving cells, which may eventually proliferate and re-establish the tumor. Accelerated repopulation—a phenomenon observed over 40 years ago and also described in human patients—is an established mechanism by which tumors respond to radiotherapy; however, little is known about the molecular mechanisms of this process. Now, in a study published in *Nature Medicine*, Chuan-Yuan Li and colleagues show that dying tumor cells use apoptosis—specifically through the functions of caspase 3, encoded by *Casp3*—to provide the initial signals to promote tumor cell repopulation.

> “...dying cells in the tumor mass stimulate the repopulation of the tumor...”

The researchers hypothesized that dying cells within the tumor provide the impetus for existing tumor cells to regrow. Li's team used bioluminescent imaging to track the growth of cells in culture and demonstrated that, following irradiation, dying cells could stimulate the growth of living tumor cells much more efficiently than non-irradiated cells. Next, they examined whether tumor cell stimulation induced by cell death could be shown *in vivo*. By injecting irradiated cells into mice, they showed that growth of irradiated tumor cells was significantly greater than non-irradiated cells. The investigators were interested in the mechanism by which this occurred and identified caspase 3 as the protease responsible for regulating tumor cell repopulation *in vitro*.

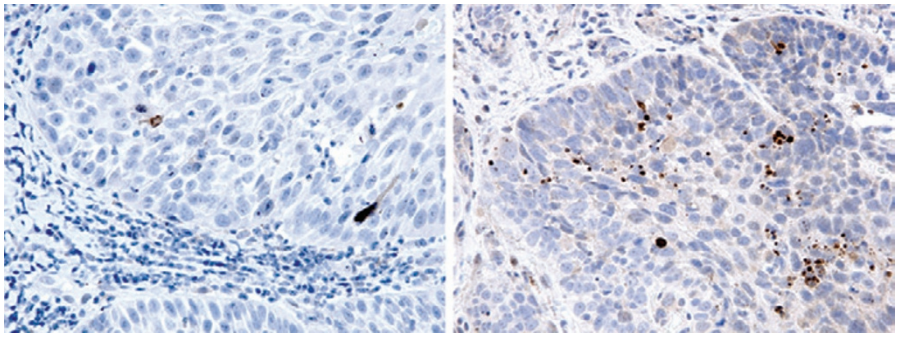

Image courtesy of C-Y. Li showing immunohistochemical staining of activated caspase 3 in samples from patients with head and neck cancer.

Li's team used a non-invasive caspase 3 reporter to monitor the activity of caspase 3 in tumor cells in mice. Using short hairpin RNA-mediated knockdown of *Casp3* expression in irradiated cells, they confirmed the involvement of caspase 3, and showed that endogenous expression of caspase 3 increased the ability of irradiated cells to promote the growth of co-seeded cells.

The relevance of caspase 3-mediated tumor repopulation was then evaluated in two patient cohorts: one in patients with head and neck cancer, the other in patients with breast cancer. In both cohorts, high levels of caspase 3 expression was associated with a higher rate of tumor recurrence or shorter survival. As Li summarizes, “we showed that dying cells in the tumor mass stimulate the repopulation of the tumor by surviving tumor cells. We also demonstrate that caspase 3, the well recognized ‘executioner’ protease during apoptosis, is a key regulator of paracrine signaling activated in dying cells to stimulate tumor repopulation. Intriguingly, and contrary to conventional wisdom, higher levels of caspase 3 in pretreatment tumor biopsies predicts a worse clinical outcome in cancer patients.”

The researchers likened their findings to observations in lower organisms, such as *Drosophila* and *Hydra* systems, in which cell death via apoptosis seems to be crucial for stimulating tissue regeneration and wound heading. The practical implications of these data are that radiotherapy efficacy might be enhanced by the use of inhibitors of caspase 3. The researchers plan to “evaluate and develop ways to enhance radiotherapy efficacy through caspase 3 inhibition, and determine whether caspase 3 might serve as a good prognosis biomarker for cancer treatment”.

Lisa Hutchinson

Original article Huang, Q. *et al*. Caspase 3–mediated stimulation of tumor cell repopulation during cancer radiotherapy. *Nat. Med.* **17**, 860–866 (2011)