Reviewer's report

**Title:** The role and prognostic value of apoptosis in colorectal carcinoma.

**Version:** 1  **Date:** 27 May 2013

**Reviewer:** Masaaki Tatsuka

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Apoptosis, a form of programmed cell-death defined morphologically, is a widely accepted biological process that is of a great interest to clinical and basic scientists who study cancer. Nowadays, there is a growing interest in the area of colorectal cancer and its management. Defects in the response to apoptosis are implicated not only in pathological aspects of colorectal cancer but also in its resistance to ionizing radiation and chemotherapeutic drugs. However, the authors in this paper demonstrated that apoptotic cells judged by TUNEL assay were frequent in samples from colorectal cancer patients with worse prognosis than good prognosis after excluding patients that had chemical treatment experiences or radiation therapy experiences. The paper is potentially interesting to know the biological aspects or physiological situation of aggressive colorectal cancers and might be valuable to establish a novel pathological diagnosis method with prognostic value.

To more better understand this paper's data, this reviewer recommends further studies as shown below.

**Major concerns:**

(1) This reviewer became concerned about which type of apoptosis is increased in aggressive colorectal cancers. For example, autophagy can be detected by immunostaining with anti-LC3B antibodies. Alternatively, mitotic catastrophe can be estimated by aberrant mitotic cells, which can be detected by immunostaining with anti-phosphorylated histone H3 antibodies. The authors' observation is possibly the result of reflecting a high frequency appearance of dead cells in aggressively growing cancer cell populations. Such an accumulation of apoptotic cells may be due to mitotic defects caused by multiple centrosomes, disorganized mitotic spindles and cytokinesis errors.

(2) This reviewer became concerned about which apoptotic signal is activated in aggressive colorectal cancers. For example, anti-caspase-9 antibodies are useful to detect the mitochondrial apoptosis signal. Anti-caspase-3 is also useful. Caspase-3 is likely to be implicated in cancer progression (Nature Medicine 17, 860–866, 2011). This reviewer wonders if it isn't time now to think of frequent caspase-3 activation states in aggressive colorectal cancers.

(3) This reviewer became concerned about which physiological situation is presented in aggressive colorectal cancers. For example, hypoxic conditions are thought to be important to progress colorectal cancers as niche, microenvironmental situation for cancer cells. Anti-HIF antibodies are available.
Minor concerns:
(1) Representative figures for judgement by TUNEL assay in each stage of colorectal cancers should be presented.
(2) The authors should mention the relationship between the apoptotic cell rate and metastasis behavior.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.