

Low dose radiation induces a highly effective p53 and immune response in follicular lymphoma

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Involved field radiation therapy with 30 to 40 Gy is a valuable local treatment for follicular lymphoma (FL) that is routinely used in clinical practice. We previously showed that, in contrast to other malignancies, very low dose radiation (2x2 Gy, days 1 and 3) is also effective, with rapid and often long lasting remissions in up to 90% of FL patients (Haas et al, JCO, 2003). However, the biological mechanism of this extreme sensitivity is not known. To study the molecular response to low dose radiation therapy in FL, gene-expression profiling using 35K spotted 60-mer oligo-arrays was performed from lymph node biopsy samples taken before treatment and 24 hours after the second dose of 2 Gy irradiation, in 15 patients. The clinical response was excellent (10 CR, 5 PR).

In all patients, a major and consistent induction of p53 target genes was seen, reflecting both proliferation arrest (e.g., *P21*, repression of cell-cycle regulated genes) and apoptosis induction (e.g., *NOXA*, *PUMA*, *BAX*, *TRAIL-R2/DR5* and *FAS*). The increase in apoptotic-related genes was confirmed by MLPA. P53 upregulation, p53-mediated proliferation arrest and apoptosis were substantiated using immunohistochemistry with dramatic increase of p53 protein levels in B-cells, less in T-cells and accessory cells, and no increase in macrophages. There was also a significant increase in the numbers of cleaved-caspase 8 positive cells (death receptor/extrinsic apoptosis pathway) with a minor increase of cleaved-caspase 9 positive cells (mitochondrial/intrinsic apoptosis pathway), suggesting a major role of the extrinsic apoptotic pathway in the hypersensitivity.

The other induced genes revealed an 'immune signature', with a whole set of biologically meaningful genes related to macrophages (e.g., *CD68*, *TLR4*), TH1 immune response (e.g., *IL18*, *CXCL9*, *10*, *11*), clearance of apoptotic cells (e.g., *C1Q*, lysosomal enzymes), tolerance (*ILT-3*, *IL-4*, *IDO*) and death receptor ligand (*TRAIL*). Immunohistochemical analysis did not show an increase in T-cell subsets and macrophages density. CD68/p53 double staining showed no increase in p53 in macrophages. These data rather suggest an activation or differentiation of resident macrophages by apoptotic cells than recruitment of novel cell populations.

This is the first global analysis of the direct molecular effect of radiotherapy and p53 related apoptosis *in vivo* in human lymphoma. Moreover, the 'immune signature' suggests that radiation-induced apoptosis in FL is not an immunologically silent process, but rather an early event that could contribute to the death and clearance of tumor cells. These insights may have important implications for modulation of the cancer-related immune response and for immunotherapeutical approaches in FL.