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Modulation of DNA repair genes induced by TLR9 agonists

A strategy to eliminate "altered" cells?

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Abbreviations: CpG-ODN, CpG-oligodeoxynucleotide; TLR9, Toll-like receptor 9

We provided evidence that the TLR9 engagement of innate immune cells present in the tumor microenvironment by CpG-oligodeoxynucleotide (CpG-ODN) induces down-modulation of DNA repair gene expression in tumor cells, sensitizing cancer cells to DNA-damaging chemotherapy. These findings expand the benefits of CpG-ODN therapy beyond induction of a strong immune response.

Synthetic oligodeoxynucleotides containing dinucleotides with unmethylated CpG motifs (CpG-ODN) are agonists of Toll-like receptor 9 (TLR9), which is expressed on cells of the immune system as well as on endothelial cells, fibroblasts, and epithelial cells.[1] In the last decade, TLR9 agonists have been included in the arsenal of anti-cancer drugs, and successes in preclinical studies using CpG-ODN and early indications of their safe use in humans have generated considerable interest in the clinical development of these agents for the treatment of cancer.[1] Experiments in murine models suggested that CpG-ODN can enhance the response to ionizing radiotherapy (RT),[2] which kills cancer by damaging DNA, and to DNA-damaging chemotherapies,[3,4] although both of these therapies induce immune suppression. We hypothesized that the success of such combinations may be related to the ability of CpG-ODN to modulate genes involved in the repair of DNA damaged by chemo- or radiotherapy. In our manuscript entitled "TLR9 agonists oppositely modulate DNA repair genes in tumor versus immune cells and enhance chemotherapy effects,"[5] we showed in a mouse model of human IGROV-1 ovarian cancer that treatment with a CpG-ODN sequence specific for murine TLR9 induce down-modulation of DNA repair genes in the tumor cells. Peritumoral injection of CpG-ODN in the peritoneal cavity was found to be critical for inducing this down-modulation. CpG-ODN did not interact directly with the human IGROV-1 cells due to the oligonucleotide's species specificity and to the lack of TLR9 expression in this cell line, raising the possibility that peritumoral TLR9-expressing cells, such as innate immune cells, and/or endothelial cells, fibroblasts and epithelial cells, induce down-regulation of DNA repair in tumor cells through a direct cell-cell interaction and/or by secreting soluble factors. The combination of cisplatin and CpG-ODN to treat IGROV-1 ovarian tumor xenografts growing in the peritoneal cavity of athymic mice induced a remarkable increase in lifespan compared with that using either reagent alone, which can be attributed to the CpG-ODN-induced modulation of a cohort of DNA repair genes that cooperatively influence the ability of cells to repair drug-induced DNA damage. The relevance of this cohort of genes in increasing sensitivity to DNA damage was analyzed on microarray data sets containing the gene expression data of ovarian[6] and breast cancer[7] samples obtained at initial cytoreductive surgery from patients who then received primary chemotherapy. Cisplatin-treated ovarian carcinoma patients as well as anthracycline-treated breast cancer patients, who showed expression levels of DNA repair genes similar to those observed in IGROV-1 xenografts treated with CpG-ODN, displayed a significantly poorer outcome compared with patients expressing DNA repair gene levels dissimilar to those of CpG-ODN-treated tumors. The combined data from our experimental and in silico analyses revealed modulation of DNA repair genes in both murine colon carcinoma cells[8] and human ovarian carcinoma cells, suggesting that this effect is not restricted to a specific cancer cell histotype.

In contrast to the down-modulation of DNA repair genes in tumor cells, in silico analyses of the effects of CpG-ODN treatment on immune cells[9] revealed essentially an upmodulation of DNA repair genes. The CpG-ODN-induced down-modulation in tumor cells and the upmodulation in immune cells might reflect a physiological

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phenomenon that occurs locally during an infectious event. Thus, upon detection of an infectious agent via TLR9, immune cells might regulate DNA repair genes to decrease their susceptibility to possible pro-apoptotic signals during infections while directly and/or indirectly inducing modulation of DNA repair genes in infected (or transformed) cells to facilitate their death (**Fig. 1**). Insignificant modulations of these genes observed in normal muscle cells suggest that CpG-ODN-activated immune cells induce down-modulation of DNA repair genes only in "altered" cells expressing apposite receptors.

Together, our data provide the first evidence that TLR9-expressing cells present in the tumor microenvironment can sensitize cancer cells to DNA-damaging chemotherapy. The opposite changes in DNA repair gene expression levels observed in immune and tumor cells point to the promise of CpG-ODN as an avenue toward improving chemotherapy treatment without increasing drug toxicity in tumor patients. Moreover, these findings confirm the importance of the intratumoral route of administration for TLR9 agonists, as we previously reported,[10] and may explain why association of CpG-ODN with DNA-damaging drugs has thus far not reached its full potential to improve clinical outcome.

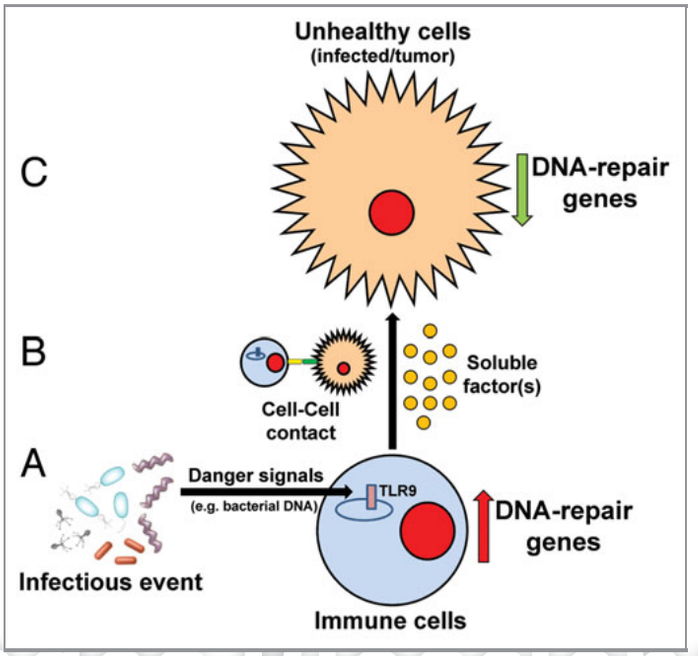

Figure 1. Hypothesized mechanisms of action of TLR9-expressing cells in modulating DNA repair genes during infections. (A) TLR9-positive cells upon detecting an infectious agent regulate DNA repair genes to decrease their susceptibility to proapoptotic signals and (B) induce modulation of DNA repair genes in infected cells (C) to facilitate their death.

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