Selective protection of normal tissues from radiation damage by activation of Toll-like receptor 5 signaling

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The toxicity of ionizing radiation (IR) is associated with the development of acute radiation syndrome primarily involving damage to the highly radiosensitive gastrointestinal (GI) tract and the hematopoietic (HP) system (1). Development of radioprotectants has been historically focused on agents that either reduce the degree of a direct damage (e.g., anti-oxidants) or stimulate tissue recovery and regeneration (e.g., cytokines) (2). These efforts have not produced a useful radiation countermeasure approved with an acceptable safety profile that would be effective against both major components of acute radiation syndrome leaving radioprotection an unmet medical need. We have recently introduced a new type of radioprotectants with a distinctly different mechanism of action which does satisfy the necessary requirements and can be considered for biodefense and medical application.

Our strategy for radioprotection is based on the observation that massive cell loss occurring in radiosensitive tissues exposed to IR occurs predominantly through apoptosis, which is almost universally suppressed in tumors. Among the variety of mechanisms acquired by tumors to avoid apoptosis, there are two most universal ones - suppression of pro-apoptotic p53 pathway and constitutive activation of pro-survival NF-kB pathway. Our approach to selective radioprotection involves the development of pharmacological agents able to activate those mechanisms of apoptosis suppression that are utilized by tumor cells in normal cells. First, we started with the development of p53 inhibitors which offered effective protection to the HP system known to suffer cell loss predominantly through the induction of p53-dependent apoptosis (3, 4). However, the pro-survival role of p53 found in GI tract (5) prevented us from using p53 inhibitors for protection from this component of acute radiation syndrome. To protect the GI tract from acute radiation syndrome, we used agents known to activate NF-kB, the pathway that is constitutively active in the majority of tumors. Specifically, we focused on NF-kB-activating factors of human intestinal microflora known to be involved in supporting viability of intestinal tissue (6). The radioprtotectant we recently described, CBLB502, was derived from bacterial (Salmonella) flagellin, the only known ligand of Toll-like receptor 5 (TLR5) which is expressed in the epithelial and endothelial cells of the intestine and presumably plays a role in the protection of the GI tract from parasitic infections. Studies with flagellin and its' less immunogenic, non-toxic derivative, CBLB502, have shown each able to provide significant radioprotection and mitigation of radiation injury to both the HP and GI systems and improve survival of mice and rhesus macaques after exposure to lethal IR (7). Importantly, CBLB502 was found effective in protecting mice from IR applied locally (head and neck),

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the regimen better imitating the use of radiation in oncology clinic.
Treatment with CBLB502 significantly enhanced expression of a number of cellular defense factors encoded by NF-κB-responsive genes such as superoxide dismutase 2 (SOD2), a well-known scavenger of reactive oxygen species, that is induced in the lamina propria of irradiated mice and primates. Furthermore, CBLB502 injection led to the induction of high levels of G-CSF and other radioprotective cytokines with only minor increase in pro-inflammatory factors, such as TNFα or IL1. Thus, the mechanism by which CBLB502 prevents radiation toxicity is multi-fold and involves mobilization of endogenous defense mechanisms, combining "traditional" radioprotective approaches (induction of antioxidants and regeneration-supporting cytokines) with suppression of apoptosis in radiosensitive cells.
CBLB502-mediated radioprotection was found to be highly selective for normal cells and did not change tumor cell sensitivity to IR regardless of whether they express functional TLR5 or not. This selectivity is presumably explained by constitutive activation of NF-kB observed in the majority of tumors (8), rendering this mechanism unsuitable for targeting by radioprotectans. The selectivity of CBLB502-mediated radioprotection for normal cells was tested in vitro by colony formation assays. Characterization of a large panel of tumor-derived and normal human and mouse cell variants grown in culture indicated that only normal cells are susceptible for radioprotection activity of CBLB502. The normal tissue selectivity of CBLB502-mediated radioprotection was further confirmed in vivo in several mouse tumor models of experimental radiotherapy, including syngenic (C57BL6 mice bearing B16 melanomas) and xenogenic tumors (athymic nude mice bearing human HCT116 colon tumors). The tumor-bearing mice were subjected to fractioned radiation treatment (3 or 4 Gy for three consecutive days, 100% lethal cumulative dose) with and without CBLB502 treatment. In all of the models tested, the antitumor effect of irradiation was accompanied by the death of all irradiated mice of the control group within 2-3 weeks after IR while treatment with CBLB502 prevented radiation-induced mortality or prolonged survival providing no protection to the tumors (Figure). These results support the development of CBLB502 as a drug able to prevent adverse effects of radiotherapy through its ability to protect both the GI tract and the HP system. The high selectivity of CBLB502 for protection of normal cells makes it uniquely qualified for radioprotection (prophylaxis) and radiomitigation regimens during cancer therapy.

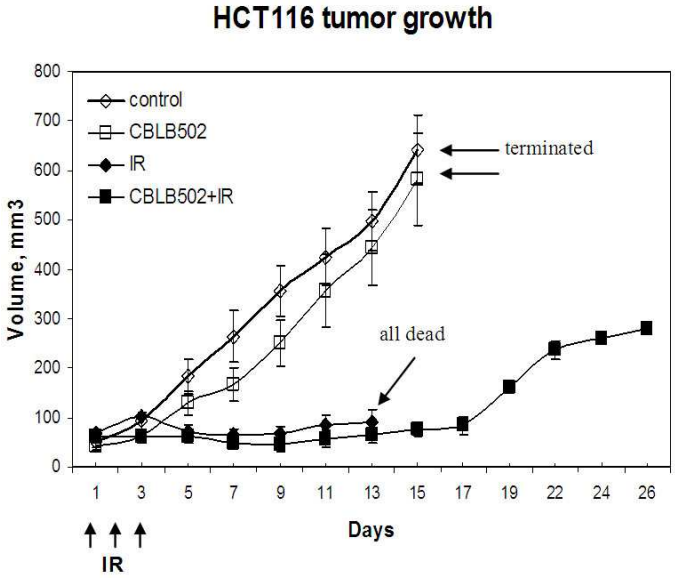

Figure. Athymic female mice were inoculated with human colorectal cancer cells HCT116. When tumors reached approximately 5 mm in diameter, mice received CBLB502 injections followed by 3.3 Gy of total body irradiation 30 min later. This treatment was repeated 3 times daily. Two other groups of mice were treated with CBLB502 alone or irradiated. Control mice received PBS injections as a vehicle control. V=π/6 x length x width x width.

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