

Commentary

Counteracting tumor radioresistance by targeting DNA repair

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The power of ionizing radiation as a therapeutic modality is based in large part on two basic principles. One is the use of sophisticated delivery systems that minimize the dose to normal tissues and maximize the dose to the tumor, an approach epitomized by the advent of intensity-modulated radiation therapy. Intensity-modulated radiation therapy harnesses sophisticated three-dimensional computer-based optimization techniques and modern imaging techniques to devise flexible treatment plans that modulate the intensity of the radiation beams across a field, providing limitless possibilities to sculpt the radiation dose and avoiding excessive doses to critical normal tissues even if the tumor has an irregular shape (1). The second principle is based on the finding that dose fractionation spares normal tissues with a slow turnover rate at the expense of tumors and normal tissues with a fast turnover. Radiation "tolerance" doses are therefore largely determined by the dose that slowly proliferating tissues in the field can tolerate; a concept that is being partially revised in light of our ability to sculpt dose distributions. In any event, the concept has arisen that tissues with slow turnover "repair" damage between dose fractions better than those with fast turnover, resulting in a therapeutic benefit, although hard evidence that this is at the level of DNA repair is annoyingly hard to obtain.

In spite of our increasing ability to selectively escalate the dose delivered to tumors, theoretical considerations suggest that the upper limit of this approach may, in many cases, still not be sufficient to overcome tumor radioresistance; *in vitro* surviving fractions of cells after 2 Gy irradiation range from 20% to >85% (2), suggesting that clinically unfeasible large fractionated doses may be

required to cure some tumors. The mechanisms underlying cellular radioresistance and identification of targets for its therapeutic manipulation are therefore of major concern in radiation oncology.

The power of ionizing radiation as a cytotoxic agent is based on its relative efficacy at causing lethal "clustered" lesions that encompass both DNA strands and that are tens of nucleotides in size. Most other double-strand breaks and other lesions are smaller and are efficiently repaired. Unrepaired double-strand breaks are lethal and mutagenic, making this process critical. At least two mechanisms, each with discrete participants, are involved in double-strand break repair. Homologous recombination uses a faithful template and is therefore only active in late S and G₂ phases of the cell cycle. Most double-strand break repairs rely on nonhomologous end-joining, which requires little sequence homology and may be inaccurate, but is still effective. Although most DNA repair proteins are expressed constitutively and in excess for the role they perform, the level of DNA damage and repair can influence intrinsic cellular radioresistance because cells with compact chromatin are more radiation-sensitive (3) and it has been known for many decades that radiosensitivity is greater in the G₂ and M phase of the cell cycle (4). Also, variants of DNA repair proteins can exist that may be clinically relevant. For example, variant Ku86 of the DNA protein kinase (DNA-PK) complex was found in patients with multiple myeloma that correlated with increased sensitivity to DNA-damaging agents including radiation (5).

However, the issue of what determines cellular radioresistance is complex. It is not only the damage that is done but the way the cell perceives that damage which is important. Central to sensing DNA double-strand breaks are ATM and other kinases that feed into well-studied DNA damage response pathways (6), indicating multiple roles for downstream events such as pathways that determine death and survival (7), proliferative signals, and those derived from the tumor microenvironment (8) in the cellular response to irradiation. The extent to which these pathways feed back to interact with DNA damage and repair is uncertain. However, adenoviral p53 transduction affects DNA repair protein expression (9), as does the epidermal growth factor receptor inhibitor Iressa (10). The interplay between DNA repair processes and other cellular events is therefore profound and there is clearly a wealth of potential targets for the biological manipulation of radiation responses. Indeed, it seems likely that the majority of biological targeting agents that are being

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assessed clinically for their anticancer efficacy will also increase the efficacy of radiation therapy. Because of the propensity of ionizing radiation to cause double-strand breaks, inhibition of these repair pathways in particular promises to enhance the efficacy and selectivity of standard tumor radiation therapy. Double-strand breaks are produced under physiologic circumstances in normal tissues, but at a very low level, and using targeted radiation therapy to selectively increase their representation within tumors in the presence of inhibitors of their repair offers a possibility for achieving a therapeutic advantage.

A variety of approaches have been used to target DNA double-strand break repair molecules for radiosensitization, including small interfering RNA, aptamers, antisense (11–14), and small-molecule inhibitors, which are being clinically developed by a number of commercial firms. In this issue, Jones et al. (15) report a novel approach to targeting nonhomologous end-joining as a means of radiosensitizing breast cancer cells. The fundamental steps in nonhomologous end-joining are recognition of double-strand breaks and processing of the termini to allow ligation and sealing of the break. The DNA-binding component Ku70/Ku80 bind DNA-PKcs to constitute DNA-PK. DNA-PK has many phosphorylation targets, one of which is Artemis, the best characterized example of a DNA-processing enzyme. Another candidate is polynucleotide kinase (16). End-processing is followed by the action of XRCC4 and DNA ligase IV as a heterocomplex that is essential for DNA resealing. Deficiency in any of the nonhomologous end-joining proteins manifests as profound radiation sensitivity, associated with a deficiency in double-strand break repair. Using an adenoviral system, the authors forced the elegant expression of a XRCC4 fragment protein comprising amino acids 115 to 292. This peptide was shown to bind ligase IV, and may block the ability of full-length XRCC4 to perform its function.

Adenovirus-mediated expression of the XRCC4 fragment resulted in radiosensitization in breast cancer cells, as measured by clonogenic survival, with a dose-modifying factor of ~1.6. The XRCC4 fragment presumably suspended nonhomologous end-joining of DNA double-strand breaks. The authors were somewhat surprised by the limited magnitude of this response. It is true that this dose modification factor is rather less than can be achieved by loss of DNA-PKcs, ATM, or Artemis. However, if repeated over a fractionated course of radiation therapy, this dose modification could still result in a quite dramatic increase in the control of radioresistant tumors. It should be said that the exact mechanism by which the XRCC4 fragment radiosensitizes cells is still not fully elucidated. XRCC4/ligase IV complex disruption was not complete. Complexes of fragments with full-length XRCC4 and ligase IV could still be isolated from peptide gene-transduced cells and it is not known whether these retained activity or whether one XRCC4 fragment within the heterocomplexes present within a double-strand break would be enough to block DNA ligation. Alternatively, the

relative lack of effect may relate to the limited influence of the XRCC4/ligase IV complex in downstream DNA damage responses. It is also worth considering alternative mechanisms, for example that the peptide might interfere with XRCC4-polynucleotide kinase complex formation, reducing the efficiency of DNA ligase IV-dependent end-joining or interact with preformed tetrameric XRCC4, excluding DNA ligase IV.

Given the plethora of potential targets within the nonhomologous end-joining group of proteins, it is worth mentioning some potential advantages and disadvantages of targeting XRCC4/ligase IV. Since its discovery in 1995 (17), the role of XRCC4 in nonhomologous end-joining has been poorly understood, although it most likely links DNA end-processing to ligation, perhaps acting as a scaffold protein (16). One advantage of targeting this process may be that most DNA repair proteins seem to be involved in diverse cellular activities in addition to repair. Thus far, this does not seem to be the case for XRCC4/ligase IV. Even the DNA ligase IV seems distinct from other DNA ligases, and the relationship with XRCC4 and DNA repair seems unique. This may give specificity to the targeting, although as mentioned, it may also decrease efficacy. Certainly, by virtue of its low abundance and possible rate-limiting nature, XRCC4/ligase IV may provide a superior target for intervention than DNA-PK or Ku proteins, which should make targeting more effective. Furthermore, cells lacking ligase IV show considerably slower rejoining kinetics following irradiation than cells lacking DNA-PKcs or Artemis, and ligase IV seems to be involved in all classes of breaks unlike the other proteins which may be more selective (18).

The idea that DNA repair is the cell's Achilles' heel that can be targeted by drugs or other means to effectively enhance responses to radiation is not new. In a sense, inhibition of DNA repair as an approach to radiosensitization may already be in use in the clinic because a number of chemotherapeutic agents that affect DNA repair have already been employed in chemoradiation protocols. Also, gene therapy approaches using thymidine kinase and gangcyclovir for radiosensitization may function through this mechanism. However, targeting the nonhomologous end-joining pathway has a certain sophistication and selectivity for radiation effects that is appealing and XRCC4/ligase IV complexes have features that make them particularly attractive targets. One would hope that the side effects of expression of XRCC4 fragments would be limited (no effects of the fragment alone on cell behavior are mentioned in the article), and in general, that these agents would be expected only to be effective in an irradiated site. However, even if this is the case, and even with intensity-modulated radiation therapy delivery, normal tissue will be within the radiation field and the incidence of complications would need to be carefully monitored. Because the nonhomologous end-joining pathway has a crucial role as a caretaker of the mammalian genome that is required in normal development, especially neurogenesis and immunity, and suppression of carcinogenesis, there is

clearly some potential risk in targeting it. As always, delivery can be used to minimize this risk. In this review, adenoviral vectors were used to deliver genes *in vitro*. Although they can also be used *in vivo*, there is no vector system currently available that involvement of could affect all, or even most cells in a tumor without involvement of a bystander effect and leakage into normal tissues. Clinically, it seems likely that small-molecule inhibitors will lead the way in this regard and it will be interesting to see what light they shed on the DNA repair process and the damage response to irradiation. Surely, there are some scientific surprises in store, and hopefully, some clinical improvement in the ability of radiation therapy to cure patients with currently resistant tumors.

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