Cell Cycle 14:20, 3207–3208; October 15, 2015; © 2015 Taylor & Francis Group, LLC

ATM serine-1981 phosphorylation is a plausible biomarker

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A biomarker has been defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] There is an unmet need for (1) biomarkers of radiation exposure following the accidental or malicious release of radioactive material, (2) predictive biomarkers of radiation toxicity in patients treated in the oncology clinic, and (3) pharmacodynamic biomarkers for DNA damaging chemotherapy and inhibitors of DNA damage signaling kinases.[2] We recently described a facile and robust assay for ATM serine-1981 phosphorylation (ATM 1981S-P).[3] Here, we consider whether ATM 1981S-P is a plausible biomarker for these unmet needs.

Ataxia telangiectasia-mutated (ATM) is a kinase activated at DNA double-strand breaks (DSBs). ATM kinase activation is associated with autophosphorylation on serine-1981.[4] ATM 1981S-P is induced in human fibroblasts by as little as 0.05 Gray (Gy) or 2 DSBs introduced using an endonuclease.[4,5] While ATM 1981S-P is not detected prior to insult, >50% of ATM molecules are phosphorylated within 15 minutes of 0.5 Gy.[4] This sensitivity promotes ATM 1981S-P as a biomarker from a technical perspective. However, this sensitivity of ATM 1981S-P may be problematic from a clinical perspective as baseline measurements may be highly dynamic and variable between patients.

ATM 1981S-P is increased in peripheral blood mononuclear cells (PBMCs) in patients after treatment with stereotactic body radiation therapy for the ablation of cancer.[6] ATM 1981S-P was increased 5-fold in a patient who received 20 Gy to a treatment volume of 15 cm^3 in 164 seconds. An approximation that assumed a constant flow of the total blood volume through the beam field suggested that each average PBMC was exposed to 0.06 Gy. ATM 1981S-P was increased 1.2-fold in a patient who received 9 Gy to a treatment volume of 10 cm^3 in 100 seconds. While it is possible that ATM 1981S-P was maximal in the latter patient prior to treatment, it is likely that there is a threshold of IR required to induce the mechanism underlying ATM kinase activation in an individual cell, which results in a quantal dose-response relationship in any given sample of PBMCs. ATM 1981S-P increases monotonically with IR and a 40-fold induction has been observed in PBMCs irradiated in whole blood with 2 Gy *ex vivo*.[6] It is not clear that this is the maximal induction of ATM 1981S-P possible. Future studies require that blood collected from patients be irradiated *ex vivo* to determine to what extent ATM 1981S-P can be induced.

The NIH Strategic Plan and Research Agenda for Medical Countermeasures Against Radiological and Nuclear Threats documents that whole-body exposure to >1 Gy IR can cause nausea and vomiting. Exposure to 1–6 Gy can damage the haematopoietic system and cause immunosuppression, infection, and death within 60 d. It is proposed that such patients could survive with medical care. ATM 1981S-P has unequaled sensitivity for the detection of radiation exposure and its dynamic range spans this dose range, suggesting that ATM 1981S-P is both biologically plausible, and likely clinically relevant.[3,4,6] The NIH has mandated that a biomarker of radiation exposure must have persistence up to 24 hour post-IR. ATM 1981S-P remains increased in fibroblasts 24 h after 2 Gy IR.[4] ATM 1981S-P is a plausible biomarker of radiation exposure.

ATM 1981S-P is also a plausible biomarker of radiation toxicity in clinical oncology. The dynamics of ATM 1981S-P between early fractions may identify patients that will develop toxicity later during their radiation therapy. A biomarker of radiation toxicity could contribute decisively to patient care by identifying patients that will be hospitalized in intensive care if steps are not taken to mitigate radiation toxicity. Since up to one third of patients receiving definitive chemoradiotherapy will be hospitalized during the course of their therapy for acute toxicities, measurement of ATM 1981S-P in the future may be a viable insurance-reimbursable biomarker for risk stratification.

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Submitted: 07/28/2015; Accepted: 08/07/2015
http://dx.doi.org/10.1080/15384101.2015.1084205

Comment on: Bakkenist CJ, et al. A quasi-quantitative dual multiplexed immunoblot method to simultaneously analyze ATM and H2AX Phosphorylation in human peripheral blood mononuclear cells. Oncoscience 2015; 2(5):542-54; PMID: 26097887

ATM 1981S-P is a plausible pharmacodynamic biomarker for DNA damaging agents. For example, ATM 1981S-P is induced in PBMCs in patients following systemic administration of the DNA damaging agent doxorubicin.[3] Ataxia telangiectasia and Rad3-related (ATR) is a kinase activated at stalled and collapsed replication forks. Pharmacologic ATM and ATR kinase inhibitors (ATRi) have been identified and these sensitize cancer cells to agents that induce DSBs and damaged replication forks, respectively. Currently, the National Cancer Institute (NCI) is supporting clinical trials of an ATRi. As ATR phosphorylates ATM serine-1981,[7] ATM 1981S-P is a simple, plausible pharmacodynamic biomarker for target engagement by ATRi in these early clinical trials. However, ATRi's induce DSBs and ATM kinase-dependent ATM 1981S-P at stalled replication forks as they cause replication fork collapse generating structures that are susceptible to endonucleases. Thus, ATRi's will inhibit and then induce ATM 1981S-P after the induction of stalled replication forks.

The clinical need for a facile and robust biomarker of DNA damage is great. Carefully designed studies may deliver ATM 1981S-P to the elite 1% of published cancer biomarkers that actually enter clinical practice.

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