Structural consequences on transforming growth factor beta-1 activation from near therapeutic X-ray doses

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Radiation therapy uses radiation doses that are far lower than those required to yield structural data. Studies of the structural impact of radiation therapy are masked by radiation damage, even in cryocooled crystals. When the structural impact is large scale, further complications result from crystal packing. Understanding the basis of cellular response to radiation is a major goal in the search for effective cancer treatments. There is growing evidence that extracellular signaling proteins orchestrate complicated behaviors between cells that collectively direct the future of tissues, representing a promising new class of targets for biomodulation. Transforming growth factor beta-1 (TGFβ-1) is one of these and in response to irradiation it initiates downstream signaling pathways that control a number of cancer related processes such as proliferation, migration, and invasion. Normally, the 25 kDa dimer of TGFβ-1 is secreted with the 55 kDa dimer, Latency associated peptide (LAP), that renders TGFβ-1 inactive, and together are known as Latent-TGFβ-1 (LTGFβ-1). Dissociation from this arrangement allows the now “activated” TGFβ-1 to bind cognate receptors that initiate signaling pathways and ultimately alter gene expression. X-ray radiation induces this activation and was first observed in the immuno-histochemical staining of irradiated mammary gland cells [1]. This work showed that radiation generated both activated TGFβ-1 and in-activatable (i.e. damaged) LTGFβ-1, suggesting two separate pathways. Reactive oxygen species (ROS) generated *in vitro* can also activate TGFβ-1 through a non-conserved methionine in LAP [2]. These studies however, overlooked the effect of X-ray generated reductive stress, which could modulate LTGFβ-1 activity through disulfide disruption, which is observed in other proteins [3].

Using solution scattering methods coupled with complementary structural techniques, we have investigated the effects of low dose X-ray radiation exposure within therapeutic dose ranges on the structural landscape of LTGFβ-1, not to study radiation damage, but to look at the structural impact of radiation therapy unbounded by the crystal lattice or artificially constrained by cryogenic conditions. We (1) characterize changes induced by radiation exposure, (2) determine protein regions most sensitive to radiation, and (3) understand the radiation chemistry that initiates the process at the therapeutic level. Our results suggest that the damage pathway results from oxidative stress and that activation is initiated but not driven by X-ray exposure [4]. LAP is revealed to be extended when unbound to TGFβ-1. These studies pave the way for a structural understanding of systems impacted by therapeutic level X-ray doses.

References:

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3. Sutton et al., *Acta Crystallogr D Biol Crystallogr,* 2013
4. Stachowski et al., *J Synch Rad,* In press