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Inside the tumor: p53 modulates calcium homeostasis

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Calcium (Ca^{2+}) is an essential element of signal transduction. Ca^{2+} signaling is involved in various cellular processes, such as proliferation and apoptosis and thus is also crucial in cancer. In particular, modulation of Ca^{2+} signaling can change cells' sensitivity to apoptotic signals, such as chemotherapeutic agents.[1]

Previous studies indicate that both tumor-suppressors (PML, PTEN, Bax) and oncogenes (Bcl-2, Ras, Akt) are able to regulate Ca^{2+} dependent apoptosis by modulating Ca^{2+} release from the endoplasmic reticulum (ER) store.[2] In turn, these factors can mediate the opening of the mitochondrial permeability transition pore.[3]

In contrast, little information was available on the transformation-related protein 53 (TRP53), better known as p53 in the regulation of Ca^{2}-dependent apoptosis. This oncosuppressor becomes activated in response to a myriad of stressors, and its activity is crucial for the regulation of cell death pathways. Since the p53s prominent pro-apoptotic role was demonstrated, the mechanism through which p53 mediates apoptosis has been a matter of intense study. Numerous publications have described the importance of p53 in both extrinsic and intrinsic apoptotic pathways.[4]

In a recent study we demonstrated that p53 is present at the ER and mitochondria-associated membranes (MAMs), where it becomes enriched upon activation. At these sites, p53 interacts with the C-terminal portion of the sarco/ER Ca^{2+}−ATPase (SERCA) pump, changing its oxidative state and, in turn, increasing ER Ca^{2+} loading. Furthermore, p53 activation enhances ER Ca^{2+} transfer to mitochondria, thus triggering pro-apoptotic mitochondrial Ca^{2+} overload and mitochondrial morphological alterations, leading to release of pro-apoptotic factors. Consistently, pharmacological p53 inactivation, as well as naturally occurring loss-of-function mutations of p53, inhibit p53s ability to increase Ca^{2+} signaling to the mitochondria, and allow for its oncogenic function.[5]

Whereas most of the mechanisms concerning intracellular Ca^{2+} handling have been successfully elucidated *in vitro*, we still know very little about the actual physiological role of these processes in the context of the tumor environment. Recent advancements in intravital imaging and genetically-encoded sensor technologies have allowed us to visualize Ca^{2+} transient changes in live mice, overcoming previous technical limitations.

In order to elucidate chemoresistance signaling and, thus, to study the effect of novel drugs on the induction of apoptosis, we investigated the role of intra-tumor Ca^{2+} signaling and apoptosis in a 3D tumor mass, by intravital fluorescence microscopy of mouse tumor models (**Fig. 1**).[6]

To that end, we generated a clone deriving from *p53*$^{-/-}$ mouse embryo fibroblasts (MEFs) transduced with H-RAS^{V12} and a clone from *p53*$^{-/-}$ MEFs transduced with H-RAS^{V12} with re-introduced wild-type p53. These clones were then transplanted to a dorsal skinfold chamber, in athymic mice to allow tumor growth. We treated the mice with aluminum phthalocyanine chloride a photosensitizer commonly used in the photodynamic therapy (PDT) of cancer. Aluminum phthalocyanine chloride accumulates in intracellular organelles, including mitochondria and ER, where it initiates the apoptotic pathway by increasing cytosolic and mitochondrial Ca^{2+} upon photoactivation.[7]

We found that p53 is necessary and sufficient for the control of Ca^{2+}-dependent apoptosis. In particular, we were able to demonstrate that Ca^{2} response induced by p53 is correlated with the ability of PDT to initiate apoptosis.

To investigate whether the observed Ca^{2+} modulation was a side effect of our genetic manipulation of p53, or whether it was a result of p53s mechanism of action, we used various pharmacological and molecular approaches to mimic p53s effects on intracellular Ca^{2+} homeostasis. Independently of the mechanism, all conditions that increased Ca^{2+} responses rescue the sensitivity to apoptosis in p53$^{-/-}$ cells, while treatments that blunted the Ca^{2+} responses in p53 wild-type background were associated with the inhibition of apoptosis as in p53$^{-/-}$ cells.[6]

Finally, the methodology used in this study is compatible with all the fluorescent probes currently available to follow intracellular parameters.

It is of the utmost importance to understand all the signaling pathways deregulated in cancer cells. Understanding the molecular mechanisms underlying the

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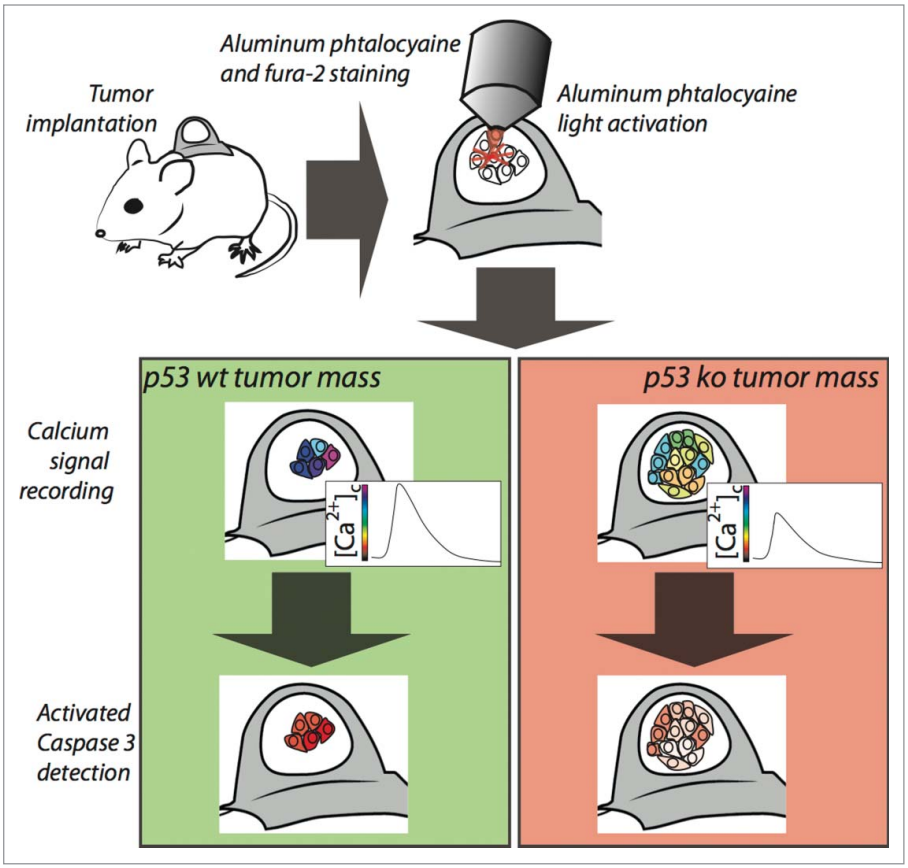

Figure 1. Imaging of calcium signaling and apoptosis into tumor mass. Tumors grown within the skinfold chamber are loaded with aluminum phtalocyanine and the Ca^{2+} indicator Fura-2. Phtalocyanine activation and Ca^{2+} signal recording are performed in the same situ using the microscope optics. After Ca^{2+} live imaging, apoptosis is measured by intravenous administration of a fluorescent marker measuring caspase activity.

regulation of tumor cell fate is a great challenge in cancer research and will provide further insights for the development of new approaches for the treatment and cure of malignancy.

The development of novel *in vivo* imaging techniques will facilitate an even greater understanding of the Ca^{2+} signal-function relationship in live organism and in tumors, and will be an invaluable tool in the identification and classification of new pharmacological targets, as well as in the optimization of current treatments.

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