

UROP Summaries

The effect of p53 on the spontaneous and exogenously induced frequency of homologous recombination in mice

Fall 2006- Present

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p53 is one of the most commonly mutated genes in human cancers.¹ It is a tumor suppressor gene that is critical for signaling cell cycle arrest or apoptosis, depending on the extent of cellular damage. If DNA damage is relatively minor, p53 signals for cell cycle arrest and DNA repair. If the damage is extensive, p53 signals for apoptosis to prevent mutations and potential tumorigenesis.¹ We set out to determine whether p53 is involved in regulating a cell's utilization of a specific form of DNA damage repair: homologous recombination (HR). HR is a critical pathway for the repair of DNA double-strand breaks (DSBs), a particularly harmful form of damage. HR repairs DSBs by using homologous sequences, such as sister chromatids, as templates.² To minimize deleterious sequence rearrangements between misaligned sequences, HR is upregulated during S phase, when the perfect template, a sister chromatid, is present.³ In addition to regular DSBs, HR also repairs one-ended DSBs that can result from replication fork breakdown, e.g., when a replication fork encounters a single-strand break or blocking DNA lesion.² Given its important role in repairing DNA damage, it is not surprising that HR is critical for maintaining genomic integrity. Misregulation of HR can lead to tumor-promoting genomic rearrangements such as loss of heterozygosity, as well as chromosomal abnormalities such as deletions or insertions. Thus, determining whether p53 status affects utilization of HR is critical for understanding one potential mechanism by which p53 prevents tumorigenesis.

In our study, we used Fluorescent Yellow Direct Repeat (FYDR) mice, which enable the detection of HR events both in vivo and in vitro. FYDR mice carry two truncated copies of the coding sequence for Enhanced Yellow Fluorescent Protein (EYFP) arranged in tandem. An HR event can restore full-length EYFP, resulting in a fluorescent cell that can easily be detected by flow cytometry and in situ imaging.^{4,5} To specifically study the effect of p53 on HR, the FYDR mice were crossed with mice that carry a deletion in p53.⁶

We were particularly interested in studying DNA repair within the pancreas, a subject that has not been widely investigated despite the high mortality rate of pancreatic cancer.⁷ To determine the effect of p53 on the spontaneous frequency of HR in vivo, pancreata from FYDR; p53 mice were analyzed by both in situ imaging and flow cytometry. In situ imaging enables the quantification of independent HR events, while flow cytometry allows for the determination of recombinant cell frequency. Our results showed no statistically significant difference between the number of HR events and the frequency of recombinant cells in p53 wild-type (WT) versus null cells, suggesting that p53 does not affect the spontaneous frequency of HR in pancreatic cells in vivo.

To further explore the effect of p53 on HR, we analyzed primary ear fibroblasts from FYDR;p53 WT and null mice in vitro. We quantified the fre-

quency of recombinant cells per cell division, which is also known as the rate of recombination. No statistically significant difference was observed in the rate of recombination in p53 WT versus null cells, which was consistent with the in vivo results. These data suggest that p53 may not have a large effect on the spontaneous rate of HR in vitro.

Since it is known that p53 plays an important role in response to DNA damage,¹ we treated cells with a DNA damaging agent (Mitomycin C) to determine the effect of p53 on the damage-induced frequency of recombination. Preliminary results from this study show that both p53 WT and null treated cells have higher frequencies of recombinant cells than control cells, indicating that Mitomycin C induces recombination events in both p53 null and WT cells. In addition, our results show that p53 WT treated cells have a statistically significantly higher frequency of recombinant cells than p53 null treated cells, suggesting that functional p53 may stimulate the repair of exogenously induced DNA damage by HR in vitro.

In summary, p53 does not appear to have an effect on the spontaneous frequency of recombinant cells in vitro or in vivo. However, p53 may affect the frequency of recombinant cells that result from the repair of exogenously induced DNA damage by HR. Thus, the effect of p53 on HR may appear only in the face of extensive DNA damage that does not exist spontaneously.

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Protease Activated Electrostatic Ligand Coating for Targeted Gene Delivery

June 2007- Present

Division of Health Sciences and Technology - Laboratory for Multiscale Regenerative Technologies (LMRT)

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Gene delivery is the focus of much research as a therapeutic tool in the treatment of diseases, including autoimmune diseases and cancer. It is an ideal therapy with which to treat patients because of the specificity of nucleic acids such as DNA to replace or restore function to diseased tissue without the systemic side effects commonly caused by modern pharmaceuticals. Non-viral, cationic, self-assembling polymer vectors have been devel-

oped to deliver nucleic acid payloads, showing great efficiency in inducing cell uptake.¹ However, these vectors are hampered by the lack of a means to effectively target and systemically deliver genes in an effective manner. The overall effectiveness of current vectors is low, as they are hindered by effects including destabilization due to hydrolysis in blood and nonspecific uptake by cells.

Researchers have added targeting ligands in an effort to address the issues of nonspecific uptake. These ligands usually consist of small peptide sequences that bind to cell surface receptors to promote specific uptake. In addition, stabilization in blood has been shown to be increased by the addition of polyethylene glycol (PEG) to provide steric hindrance, which helps the nanoparticle polyplex evade uptake by the mononuclear phagocyte system and improve accumulation in the tumor. Investigators at the Laboratory for Multiscale Regenerative Technologies (LMRT) previously developed a novel approach for the bioconjugation of a protease cleavable ligand with PEG that is activated in the presence of MMP-2, an enzyme that is upregulated in tumor angiogenesis. This bioconjugated polymer considerably increases circulation time and may be proteolytically cleaved to expose cloaked functions, such as a targeting ligand for specific cell uptake.²

Our current study adapts and synthesizes a modified protease cleavable peptide-PEG polymer that works in conjunction with a biodegradable, cationic nucleic acid condensing vector, conferring this cationic polymer with increased circulation times and passive tumor targeting ability.³ Our goals include the optimization of the nanoparticle formulation in vitro and the analysis of its circulation and localization properties in vivo with GFP as the transfection payload. Preliminary results are promising as we continue to refine our modular nanoparticle design. While currently optimized for DNA delivery, our system is general enough for adaptation to siRNA delivery, and will thereby provide new avenues for the treatment of disease through gene therapy.

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Structure and Function of MEG3

June 2007- Present

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Human pituitary adenomas comprise roughly 10% of all diagnosed brain tumors and affect the levels of hormones produced by the pituitary gland. These tumors can arise from any of the six types of cells found in the pituitary gland: corticotrophs, somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and null cells. Pituitary tumors affect hormone production by either decreasing or increasing the level of hormones. These hormones include prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and growth hormone (GH). Some adenomas are nonfunctional and do not produce any hormones; hence, they are often referred to as null-cell adeno-

mas. Approximately 25-30% of pituitary adenomas are nonfunctional, while the rest are functional and can cause clinical disorders. For example, the overproduction of growth hormone or somatotrophs can result in gigantism in children and acromegaly in adults. Similarly, high levels of ACTH in the pituitary gland can cause Cushing's disease. However, Cushing's disease is sometimes independent of ACTH levels and can be affected solely by the level of cortisol in the adrenal glands. Since pituitary adenomas can have significant effects on patients and there is currently no effective medical therapy for these tumors, it is critical for scientists to examine their genesis and development so as to find an effective way to suppress the growth of these tumors.

In previous studies conducted at Massachusetts General Hospital's (MGH) Neuroendocrine Unit, a maternally-expressed imprinted gene called MEG3, located on chromosome 14q.32.2, was found to be a potential growth suppressor in tumor cells. A study conducted at MGH by Dr. Xun Zhang et al. showed through cDNA-representational difference analysis that MEG3 was expressed in normal human gonadotrophs, but not in non-functional pituitary tumors. Real-time PCR (RT-PCR) confirmed that a MEG3 isoform, MEG3a, was not expressed in growth-hormone-secreting and nonfunctional pituitary tumors. These results suggested that MEG3 might be involved in controlling rates of cell proliferation.

To test this hypothesis, the cDNA for MEG3a was cloned into the mammalian vector pCI-neo and transfected into several human cancer cell lines that previously did not show MEG3 expression. The transfection reduced the cell clone numbers in these cancer cell lines by approximately 70%, thus confirming the hypothesis that MEG3 inhibits cell proliferation. In another study conducted at MGH by Dr. Jing Zhao et al., MEG3 loss in human tumors was found to be associated with hypermethylation of the promoter region. DNA from normal and tumor pituitary samples was treated with sodium

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bisulfite and then analyzed for methylation patterns in its promoter region. The majority of tumor DNA showed high levels of methylation in its promoter region, while normal pituitary DNA was unmethylated. Furthermore, a methylation inhibitor was used to demethylate genomic DNA from the human cancer cell line, MCF7, and this treatment restored MEG3 expression. These results further confirmed that MEG3 plays a key role in the pathogenesis of human tumors.

In the present study, I am working with another researcher at MGH, Dr. Dalia Batista, on several different kinds of pituitary tumors in order to derive their relationship to MEG3 expression. We are investigating corticotropin-secreting adenomas, somatotropin-secreting adenomas, prolactinomas, and non-functioning tumors. Our goals are to discover the structural and functional aspects of MEG3 in these pituitary tumors, to understand the mechanism for loss of MEG3 expression, and to study the effects of MEG3 on growth and hormone production in these tumors. In situ hybridization and immunohistochemical staining will be employed to determine which kinds of pituitary cells express MEG3. Real-time (RT) PCR will then be performed to examine the expression of MEG3 in pituitary tumors, and to determine if a loss of heterozygosity due to a missing allele in the MEG3 gene inhibits MEG3 manifestation in pituitary tumors. Furthermore, bisulfite treatment of DNA, followed by PCR amplification of the promoter region and DNA sequencing, will be used to analyze the methylation patterns of DNA from pituitary tumors. Thus, we will determine whether hypermethylation is responsible for the loss of MEG3 expression in pituitary tumor cells. Later in the experiment, we will transfect pituitary tumor cells with MEG3 in order to study its effect on their growth.

Since there are no effective therapies available, most tumor patients must resort to surgery or radiation therapy. The results of this project will hopefully shed light on the mechanisms underlying pituitary tumorigenesis and provide insight on targeted-therapeutic approaches for the treatment of human pituitary tumors.

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Detection of Nanoparticle Assembly Using Suspended Microchannel Resonators

January 2007 – Present

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The Nanoscale Sensing Group in the MIT Media Lab recently developed fluid-filled suspended microchannel resonators (SMRs) to measure the masses of biomolecules, single cells, and single nanoparticles in fluid.¹ These devices use the basic principle of cantilever detection: changes in the cantilever's resonant frequency are related to the mass of nano- or micro-scale compounds passing within or binding to the channel, enabling

continuous, frequency-based observation of mass changes (Δm) in the cantilever. The frequency dependence on the cantilever mass is described by the equation below.

$$f = \left(\frac{1}{2\pi} \right) \sqrt{\frac{k}{m^* + \alpha \Delta m}}$$

Equation 1: “f” is the frequency in Hz, “ Δm ” is the additional mass added to the cantilever, “k” is the spring constant, “ m^* ” is the effective mass, and “ α ” is a constant dependent on the geometry of the added mass.

We hypothesized that SMRs could be used to sense the assembly of nanoparticles, mediated by virus-sized polystyrene beads. Spherical gold nanoparticles (NPs) were used in the assembly, as well as polystyrene beads with streptavidin proteins on their surfaces for binding to biotin.³ A polyethylene glycol polymer (biotin-PEG-thiol) expressing a biotin on one end and a thiol on the other was synthesized for creating a stable, complementary coating on the gold NPs. These polymer coatings were optimized for stability and ligand-mediated biotin-streptavidin assembly was monitored using dynamic light scattering and spectrophotometry. Finally, the assembly of gold NPs and beads was measured with SMRs. Mass histograms demonstrated that over 70% of the original gold NP monomers were able to coalesce through biotin-streptavidin binding into mass aggregates detected by the SMRs. These initial results provide evidence that mass-mediated detection of NP assembly could be used in the future to sense assembly around a variety of pathogenic targets (i.e., viruses, bacteria, etc.) in specimens not amenable to optical analysis.

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