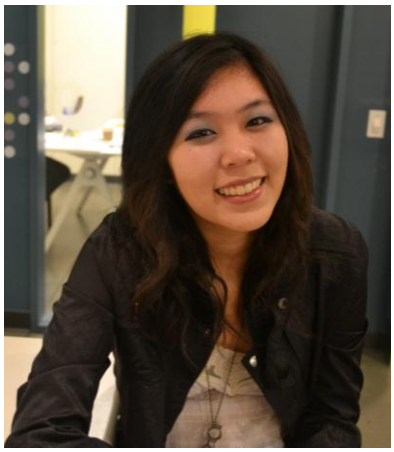
Could apoptotic genes play a part in cancer biology and treatment?

By: Amita Risbud, Junior, Health and Humanity and Biological Sciences Double Major



Apoptotic genes

It has been recently understood that genes that regulate apoptosis in an organism have a significant impact on malignant tumor development. Apoptosis is the regular process by which cells are programmed to die and allow for new growths. Should apoptosis be disrupted, cells can proliferate rapidly without any destruction of old cells, and a tumor can develop. Some oncogenic mutations have been found to interfere with apoptosis, which poses a tremendous risk for cancer. (More on current information on apoptosis and its link to cancer can be found here.)

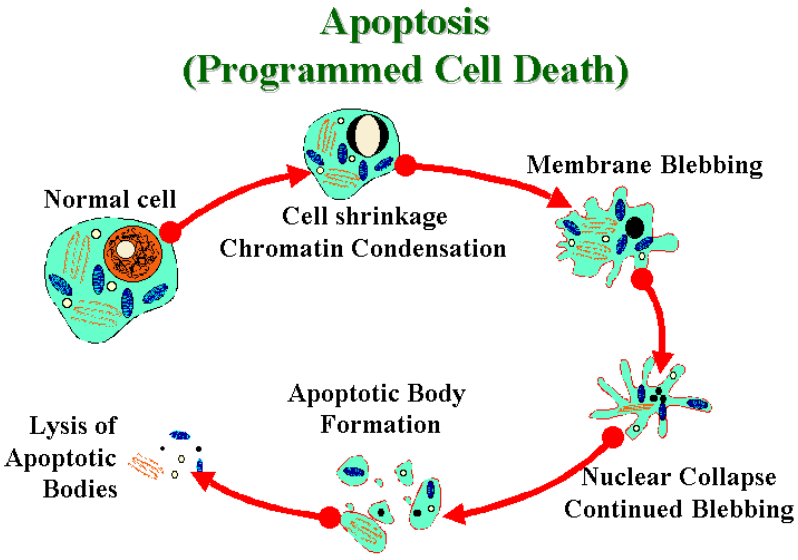


Figure 1. A simplified representation of apoptosis. (http://1.bp.blogspot.com/-LMyk8uUjo8c/Tk9vibMiKeI/AAAAAAAAAEA/i4rSNRK8XaA/s1600/Apoptosis.gif)

USC undergraduate junior Tiffany Li has been studying the gene expression of various apoptotic genes (namely, p21, TP53, and Rev1) under Dr. Valter D. Longo at the Davis School of Gerontology on the University Park Campus. Dr. Longo is the director of Longevity Institute within the School of Gerontology, as well as a professor of gerontology and biology at USC.

Researcher Background

Tiffany Li is part of the prestigious Trojan Admission Pre-Pharmacy (TAP) Program, which has allowed her to complete an undergraduate bachelor's degree in three years with guaranteed admission to the USC School of Pharmacy in the following year. Originally from Fremont, CA, Li will graduate this spring with a degree in Biological Sciences.

Although she was never compelled to pursue research positions for resume-building and competitive application to pharmacy schools, Li shares that she took up this opportunity because she found research to be a constructive extracurricular activity and a great experience for a pre-health student. She initially sought labs conducting research on disease-related topics, because she saw value in being in exposed to research where pharmaceuticals might be useful. She then joined Dr. Longo's lab in the beginning of her sophomore year. Dr. Longo's research caught her interest because it was especially relevant to her coursework at the time: the molecular studies in Dr. Longo's lab certainly enhanced her lab skills in her molecular biology lab. As Li became more comfortable with basic procedures in the lab, she was assigned a project under graduate student Hong-Seok Shim. She shares that more independent work has helped her build confidence and leadership in a laboratory setting.

Methods

Li uses real-time PCR analysis on rat tissue (usually from starved muscle or liver samples). Adhering to the real-time PCR protocol, she first breaks tissue samples into smaller pieces and homogenizes them in TRI reagent solution, which eventually helps isolate DNA, RNA, and proteins from the tissue. Centrifuge and subsequent exposure to isopropanol, ethanol, and other chemicals facilitates RNA extraction and removal of extraneous tissue components. The RNA, under proper conditions, is expected to develop into a pellet.

The pellet is re-suspended in a combination of buffers before conducting spectrophotometry to measure the RNA yield. The concentration of RNA solution is determined by measuring absorbance levels of just a few drops of the sample at 260 mm.

The RNA sample is then enhanced with primers and DEPC water (purified water that minimizes contamination) in varying amounts, depending on the concentrations measured by the spectrophotometer. Heat incubation melts the secondary structure of the RNA. cDNA is then isolated by cooling the RNA sample with additional buffer, dNTPs, and reverse transcriptase, and then it is stored at -20 ˚C. DNA is a preferred form because it is more stable than RNA.

In the final step of PCR analysis, Li adds buffer, dNTPs, GoTaq polymerase, primers, ddH2O, and SYBR green, a bright dye that helps identify different fragments of the DNA.

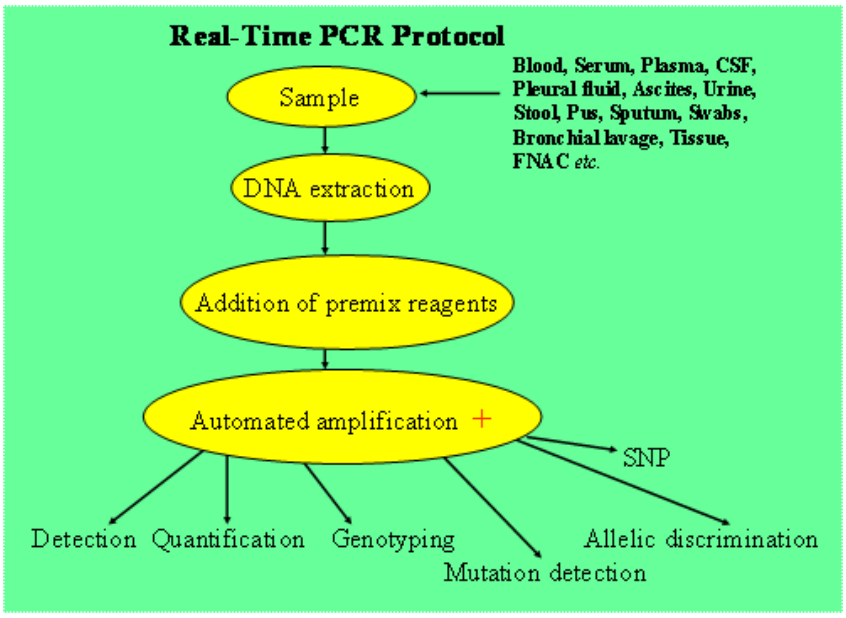


Figure 2. A general protocol for real-time PCR.
(http://www.diagnosticsgenome.com/image/real-time-pcr_clip.gif)

Current Data

The lab compares the expression of various apoptotic genes and how some may be more responsive to starvation conditions, and whether they may be linked to the occurrence of cancer. Previous measurements of expression in genes Puma, Bax, and Noxa are shown in the graphs below.

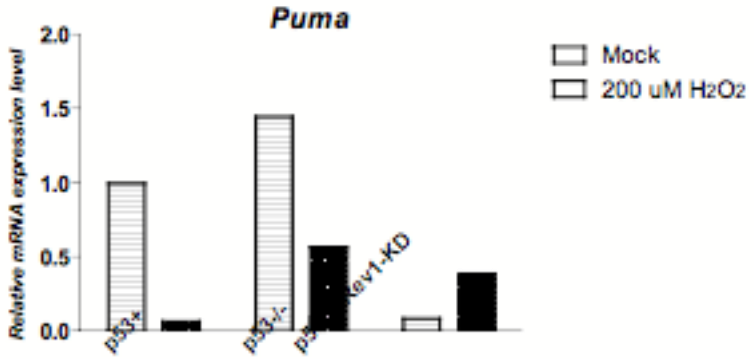


Figure 3. Expression of various samples of Puma gene. (11/08/2012)

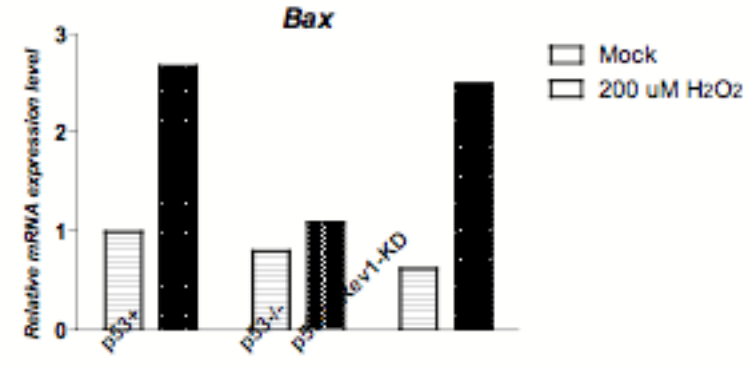


Figure 4. Expression of various samples of Bax gene. (11/30/2012)

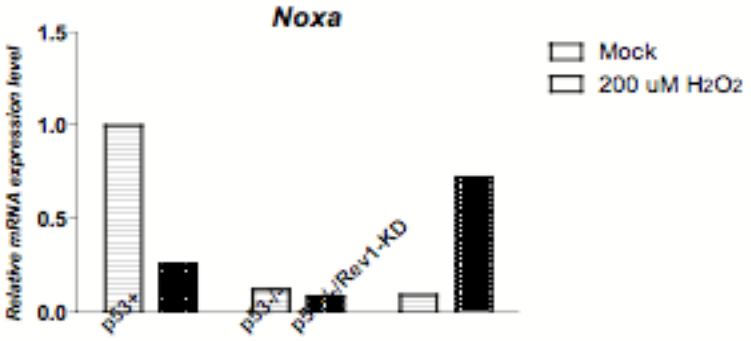


Figure 5. Expression of various samples of Noxa gene (11/15/2012)

Li has been responsible primarily for studying the expression of apoptotic genes p21 and p53 from liver samples in mice. Data up to date show significantly greater expression of p53 than p21 in starved cells, as shown by the graphs below. The lab is now looking into *why* one gene may be more affected by the starved state, and whether this might explain any link between cancer and starvation conditions.

Future Implications

Li notes that her lab's research should contribute to a greater understanding of the mechanism of gene expression in cancer cells, and how cancer interacts with the apoptotic genes that Dr. Longo studies. Some current research has suggested there are some mutations that enhance apoptosis, which could inhibit carcinogenesis, and there are others that interfere with apoptosis, thereby facilitating tumorigenesis. More extensive study of various mutations' stimulatory effects on apoptotic genes may help scientists discover ways to combat those mutations that actually advance tumor growth.

Present studies inform that cancer-fighting drugs may successfully promote apoptosis, but as a side effect, still induce harmful cell death elsewhere. There could be serious impacts on stress response and general well-being. Interpreting the molecular basis of cancer proliferation more closely should certainly help researchers develop effective gene therapies to prevent or treat cancer in the future.