AN INTERVIEW WITH JIM HOERTER

Principal Investigator

National Institutes of Health AREA Grant ($347,000)

STEM CELLS AND MELANOMA

Award Period: 2010-2013

HOW DOES YOUR RESEARCH RELATE TO TEACHING?

I consider my research lab as an extension of the classroom. It is an ideal place for teaching and learning. It's an excellent environment to help students learn biology by designing experiments and discovering new knowledge about how life works. Carrying out a research project is one of the most valuable learning experiences that a student can have. As a professional educator, I believe I should be engaged in advancing knowledge in my discipline. If I can involve students in this process, they become partners in scholarship. Students are co-authors on my manuscripts, present seminars and design experiments. We work together as a team.

I want to dispel the common notion that education and research are separate activities, and that research can only occur at a graduate university. I believe research and education go hand in hand. The classroom is not the only place where learning happens, in fact, it is taking place much more outside of the classroom than inside the classroom these days. The National Institutes of Health which is providing funds for this research recognizes how important it is to have undergraduates take an important role in this research. More often than not, this affects their career decisions in a very positive way.

WHAT ARE YOUR MAJOR RESEARCH INTERESTS?

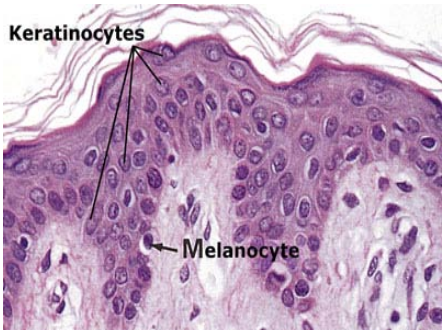
My major research right now is on how our body uses adult stem cells to repair damaged tissue. This fascinates me. I want to learn how adult stems are regulated in the body. I also want to understand how they are protected from DNA damage, and how they repair themselves. I am using the melanocyte stem cell in the zebrafish as a model to understand how all adult stem cells in the body are regulated and repaired.

Understanding how adult stem cells are regulated is a foundation for the field of regenerative medicine or the potential use of adult stem cells to repair or regenerate damage tissue in the human body. Zebrafish, unlike humans, can regenerate heart and retinal tissue by using their own adult stem cells. It would be wonderful if we could learn how to turn on adult stem cells in the human body so they could naturally repair or regenerate damaged hearts, or replace a retina and restore sight to people who are blind.

HOW DID YOU GET INTERESTED IN STEM CELLS?

I have always been interested in the genetic control of development. It all started many years ago in graduate school at Pennsylvania State University when I decided to focus on the development of the melanocyte because it was so easy to identify and trace its development. The melanocyte contains a natural pigment called melanin, making it easy to identify without staining. Since then, I have always been interested in understanding how this cell becomes wayward, producing a tumor called melanoma. I always used cell culture in my research, but during my recent Fulbright research sabbatical in Dublin, Ireland (2008), I became fascinated with using zebrafish to study the role of stem cells in the development of melanoma. When I returned home, I immediately started making plans to use this model system to answer some important questions about the origins of melanoma.

WHAT ROLE DO MELANOCYTES PLAY IN THE HUMAN BODY?



Melanocytes are melanin producing cells located on the basement membrane of the skin's dermal layer. When this pigment is transferred to the keratinocytes of the epidermis, it leads to a "tan", protecting the skin from the rays of the sun. Keratinocytes eventually die and are lost from the skin, pigment is lost and your tan fades.

WHAT IS A MELANOCYTE STEM CELL?

Adult melanocytes have a very long lifespan, but occasionally must be replaced as they become damaged or worn out. Melanocyte stem cells in the dermis are then signaled to produce new melanocytes. Just until recently, melanocyte stem cells were thought to only exist in the hair follicle. Now they have been identified in the lining of nerves in the dermis of the skin. These stem cells are kept in reserve and are only used when signaled to replace a worn out or damaged melanocyte.

IS MELANOMA A SERIOUS DISEASE?

Melanoma is the most deadly form of human skin cancer. Over the past several decades, the incidence of melanoma has steadily risen in the United State with about 60,000 cases and 8,000 deaths reported each year. Melanoma is very aggressive when it becomes malignant. Sunlight exposure is a major contributor to melanoma. People are spending more time outdoors and exposing their skin to high intensity tanning bed radiation. It is important that we understand how sunlight causes cancer so we can develop better sunscreens. Understanding the early events in the development of melanoma will permit earlier detection and development of more effective drugs to treat and prevent its reoccurrence.

WHAT IS THE MAJOR OBJECTIVE OF YOUR RESEARCH?

Although solar UV radiation plays a leading role in melanoma, it is still not clear how it initiates melanoma. The long-standing hypothesis is that mature melanocytes accumulate mutations over time until critical genes are damaged and the melanocyte becomes cancerous, forming an aggressive tumor.

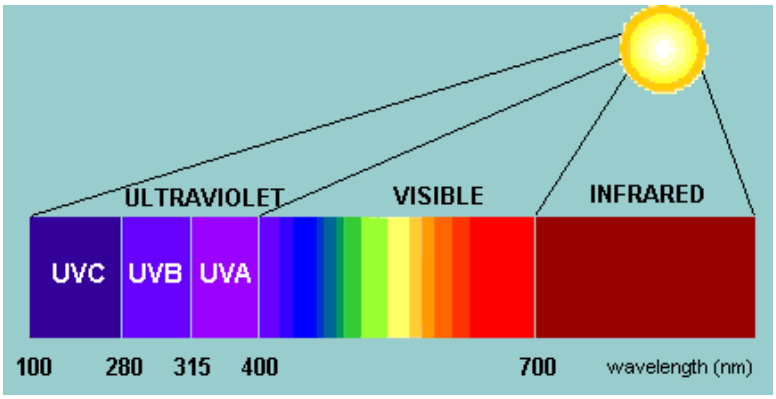
My research project will investigate an alternative hypothesis that melanoma really begins in a solar-damaged melanocyte stem cell in the dermis of the skin. My hypothesis is that over many years of solar exposure, a melanocyte stem cell accumulates mutations. Then if this melanocyte stem cell is called upon at some future time to replace a worn-out or damaged adult melanocyte in the skin, it will contain these mutations and thus be more susceptible to becoming cancerous upon further exposure to sunlight. Melanocytes exposed to UVB/UVA at different stages of development will give forth different forms of melanoma ranging from invasive malignant melanoma to isolated atypical epidermal melanocytes or moles. The recent discovery of melanocyte stem cells in the dermis of the skin makes this hypothesis even more compelling. This makes real sense when you know that severe childhood sunburn increases the chances of developing melanoma later in life. The stem cells get damaged, and if not

repaired, can be passed on to regenerated melanocytes later in life, making them more susceptible to subsequent solar radiation.

WHAT IS THE DIFFERENCE BETWEEN SOLAR UVA AND UVB?

UV radiation can be divided into three wavelength ranges according to their photochemistry: UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm). The stratospheric ozone absorbs all UVC radiation before it reaches the earth's surface so we really do not have to be concerned about it when we walk outside. However, the ozone layer does not absorb all the UVB radiation. All sunlight contains some UVB radiation. UVB radiation is responsible for causing sunburn. Furthermore, reductions in stratospheric ozone levels due to air pollution lead to higher levels of UVB reaching the Earth's surface. In contrast, all of the solar UVA radiation gets through the ozone layer and reaches the Earth's surface. It is important to remember that unlike UVB, UVA radiation can pass through auto and window glass as well as eyeglass lenses. Many sunscreens block UVB but not UVA.

WHAT ARE THE HEALTH RISKS OF INCREASED EXPOSURE TO SOLAR UVA AND UVB?



UVB causes non-melanoma skin cancer and plays a major role in malignant melanoma development. In addition, UVB has been linked to cataracts -- a clouding of the eye's lens. The role of UVA radiation in skin cancer is more controversial, but recent studies suggest that it plays an important role in melanoma. There is a higher incidence of skin cancer among individuals who visit tanning beds which use UVA and UVB to induce the tanning response. It should be kept in mind that sunlight exposure should not be avoided all together. There are some very beneficial effects of sunlight such as the production of vitamin D. In fact, the overuse of cosmetics and lotions with sunscreens has actually increased the number of people who are vitamin D deficient. Deficiency of vitamin D has been linked with all kinds of cancers. So sunlight is like a two-edged sword, it good for our health, but too much can lead to serious diseases.

WHY DID YOU DECIDE TO USE ZEBRAFISH AS YOUR EXPERIMENTAL ANIMAL?

Zebrafish are ideal organisms to study human cancer. They are easy to breed and maintain, and you can rear large numbers for studies rather cheaply. Zebrafish genes which control melanocyte development are very similar to mammals. In fact 80% of the genes in zebrafish are similar to human genes! Another important advantage of zebrafish is the availability of unique strains. Zebrafish have been produced using genetic engineering that contains human genes known to be damaged in melanoma such as BRAF and NRAS. I can use these strains to see if these mutations will make the stem cells more susceptible to sunlight-induced melanoma.

HOW CAN YOU BE SURE YOU ARE IRRADIATING MELANOCYTE STEM CELLS?

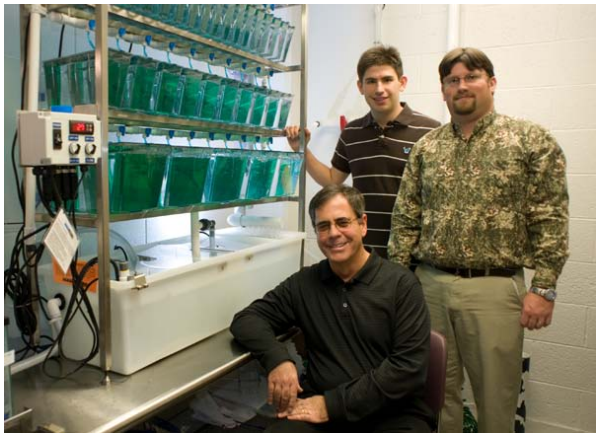
My experimental plan will utilize a recently discovered method for eliminating all adult melanocytes but not melanocyte stem cells in zebrafish, permitting us to irradiate only the melanocyte stem cell population and then observing the effects on the regenerated melanocytes derived from these damaged stem cells. The entire melanocyte population is regenerated within 4 weeks after drug washout; even the exact stripe pattern is restored. We can eliminate the adult melanocytes and induce regeneration many times, so we can actually observe the effects of multiple rounds of irradiation of melanocyte stem cells on the regenerated adult melanocyte population.

Zebrafish are ideal for research on the role of stem cells in cancer. A harmless chemical can be added to the water eliminate adult melanocytes but not melanocyte stem cells.

In 4 weeks, melanocyte stem cells regenerate the entire adult melanocyte population, restoring the original pigment stripped pattern. Any unrepaired damage in the stem cells will be passed on to the adult melanocytes.

HOW DO YOU MAINTAIN SUCH LARGE NUMBERS OF ZEBRAFISH?

A Merit Foundation Award from Ferris provided funds to purchase a zebrafish habitat to start a zebrafish research colony. This is now located in the Animal Care Facility in the Science Building. It is a really nice system that cleans and disinfects the water to prevent spread of disease. The water temperature is maintained at 28°C. Zebrafish are maintained with a 14:10 h light/dark cycle and fed living brine shrimp twice per day. It wasn't easy getting this habitat to work correctly, and it took a lot of trial and error to figure out how to breed the fish. Thanks to Richard Marble, Animal Care Facility Directory, we finally got things worked out. With the NIH grant award, we are getting an additional unit, so we should have hundreds and hundreds of fish available for our experiments.

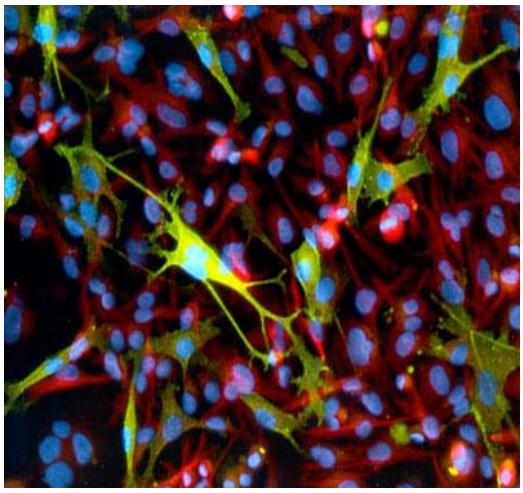


Ryan Freye (standing left) is a pharmacy student at the university. He learned all the procedures for zebrafish breeding and rearing, and took a major role in performing and documenting the fish radiation protocols. Richard Marble (standing right), Director of the Animal Care Facility, has overall responsibility for the management of the zebrafish colony. Jim Hoerter (seated) is P.I. for the research project.

HOW DO YOU EXPOSE THE FISH TO SOLAR RADIATION?

All zebrafish experiments are performed in accordance to regulatory standards as outlined by the Institutional Animal Care and Use Committee (IACUC) at Ferris State University. The melanocyte stem cells in the zebrafish are exposed to multiple rounds of UVA and UVB using a circular radiation chamber. Zebrafish are kept in the dark 24 hrs. before and 24 hrs. after the radiation treatment to prevent activation of a DNA repair mechanism not found in humans.

HOW WILL YOU KNOW IF THE TUMORS ARE MELANOMA?



Our next stage of experiments will be examining these tumors by slicing them up into very thin sections and then looking for specific biomarkers that characterize the different stages of melanoma. We use a very powerful method called fluorescence microscopy to identify these biomarkers. I had good success with this method while I was a visiting Fulbright Research Professor at Dublin Institute of Technology. Biomarkers "glow" when they are present in the tumor sample.

DO YOU HAVE ANY OTHER PROJECTS?

Yes, I am so very excited about zebrafish and how they can be used in the teaching lab that I submitted an NSF grant to establish a national zebrafish education and research network (ZERN) to help coordinate innovative uses for zebrafish in the classroom. They are wonderful for teaching important biological concepts and the scientific method to undergraduate biology students. When we do this, students are able to see how biologists work to gain knowledge about how life works.