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Article #5

**Identifying Genes Involved in
Suppression of Tumor Formation in
the Planarian *Schmidtea mediterranea***

ERIN DORSTEN

BIO 4020-02: Current Literature: Biology of Regeneration

Fall 2014

Dr. Labib Rouhana

Dr. Rouhana notes that students were challenged to write a scientific proposal centered in the study of regenerative organisms. This proposal describes experiments designed to identify genes involved in protecting an organism with negligible senescence from tumor formation. Ms. Dorsten was able to formulate a proposal of outstanding quality with regards to both literary competency and scientific creativity. Her work is written in clear and concise “stand-alone” statements with smooth transitions within paragraphs and throughout the proposal. Exemplary attention to detail is evident by the use of correct use of complex scientific terminology throughout this work.

BACKGROUND

Planarians, flatworms widely known for their incredible regenerative capabilities, are able to restore an entire organism from even a small fragment of tissue. This ability to regenerate is attributed solely to neoblasts, pluripotent adult stem cells located throughout the parenchyma of the animal (Newmark and Sanchez Alvarado, 2002). Neoblasts are stimulated to migrate and proliferate in times of injury (Guedelhofer and Sanchez Alvarado, 2012). Lethally irradiated planarians (devoid of stem cells and therefore unable to regenerate) can restore regenerative capability through transplantation of a single neoblast from a healthy planarian (Wagner et al., 2011). Many studies have concluded that the population of neoblasts is not homogenous (Scimone et al., 2014), and there are different responses to different injury types. Wenemoser and Reddien (2012) observed a body-wide increase in mitotic activity, such as cell division and migration, with any injury.

A second peak was localized at the wound site and observed only in those planarians where tissue was lost during injury. Planarians have also been found to be equipped to handle not only injury but microorganisms as well. Various bacterial strains that are pathogenic to humans were cleared from their tissue, including *Legionella pneumophila* and *Mycobacterium tuberculosis* among others (Abnave et al., 2014). Through RNA sequencing, the researchers were able to identify a specific gene utilized by the planarian during clearance of bacteria from tissues (Abnave et al., 2014). Since planarians are able to resist infection of bacterial agents and restore normal regeneration once the bacteria has been cleared (Abnave et al., 2014), questions involving the resistance to more serious diseases, such as cancer, follow. For example, would a planarian be able to recognize cancerous cells, activate appropriate signaling pathways, and correctly execute the mechanisms needed to rid the animal of the abnormalities?

Cancer, the second leading cause of death in the United States, accounts for 1 of every 4 deaths (American Cancer Society, 2014). In

simplest terms, cancer is an out-of-control division of abnormal cells (American Cancer Society, 2014). In many forms of cancer, the abnormal cells group together to form a tumor which can replace healthy tissue (American Cancer Society, 2014). Depending on where the tumor is located and how large it has grown, it can interrupt normal functioning of various organs and cause pain, weakness, and/or fever (American Cancer Society, 2014). Cancer can be caused by genetics along with a wide variety of activities including UV exposure and tobacco use (American Cancer Society, 2014). Planarians routinely employ above-average proliferation of neoblasts in response to changes in homeostasis. Therefore, planarians should theoretically be at a high risk of developing cancerous tumors. However, various studies have brought the concept of tumor suppression in regenerative animals to the surface and numerous genes involved have been identified (Pearson and Sanchez Alvarado, 2008; Pearson and Sanchez Alvarado, 2009; Sanchez Alvarado, 2012).

SPECIFIC AIMS

Initially, the goal of the proposed research is to confirm the presence of tumor-suppressing genes in planarians and determine how effective these genes are in suppressing tumor formation at varying levels of carcinogenic administration. The next goal is to determine whether or not silencing of these tumor-suppressing genes makes planarian more susceptible to the formation of tumors. This experiment will help to determine if mechanisms other than the previously found genes, such as p53 (Pearson and Sanchez Alvarado, 2009), are involved in suppressing tumors in highly regenerative animals. Lastly, this research aspires to determine whether orthologs of these tumor-suppressing genes exist in humans. It can be hypothesized that planarians utilized in this experiment will display these previously found tumor-suppressing genes, silencing these genes will lead to increased risk of tumor formation, and these genes will be conserved in humans to some degree. Overall, the goal of this research is to attempt to develop new avenues for anti-cancer therapies for individuals at a high risk of developing cancer.

RESEARCH DESIGN

In order to accomplish the first goal of confirming the presence of tumor-suppressing genes, an experiment composed of four different groups of the planarian *Schmidtea mediterranea* will be performed.

Group 1 will be a control group of healthy, intact planarians that act as a baseline for all measurements taken.

Group 2 will be intact planarians that are given one of three carcinogens, 3,4- dibenzpyrene, DDT, or 1,2:5,6-dibenzanthracene (Schaeffer, 1993), in step-wise increases. The use of multiple carcinogens will assist in determining the changes in gene expression due to administration of a chemical versus changes in response to cancer.

Group 3 will be planarians that are amputated and allowed to begin regeneration before administration of the same carcinogens as in the second group in step-wise increases to determine if the increased presence and proliferation of neoblasts affects gene expression.

Group 4 will be planarians that are lethally irradiated and administered carcinogens in step-wise increases as in groups 2 and 3.

This last group will be able to confirm or refute whether the presence of neoblasts affects tumor suppression. Since neoblasts are the only proliferative cells within planarians, it can be hypothesized that they are the main regulators of proliferation. Therefore, irradiated planarians are not expected to form tumors.

A carcinogen is one of any number of agents that cause cancer by affecting the DNA of a cell (*Encyclopedia Britannica*, 2014). Various compounds have been identified that induce tumor growth within planarians (Schaeffer, 1993), and these compounds can be administered through feeding or transdermal application. The

carcinogens used will be examined before administration in order to determine the mutation they will cause on the DNA of the planarian. Therefore, visualization of mutated DNA through DNA visualization software may be utilized. All planarians, except those in the control group, will be administered the same concentration of their respective carcinogen on day 1. The planarians will be given a sufficient amount of time for the carcinogen to mutate the DNA of a number of cells, and these cells will be allowed to replicate the DNA. At this time point, a subset of planarians from groups 2, 3, and 4 will be fixed and subjected to *in situ* hybridization for *Smed-p53*. If no DNA was mutated, and therefore no tumors can be formed, the process will be repeated with an increased concentration of carcinogens administered to the remaining planarians. This cycle will continue until a tumor is observed in each group. At each time point, a number of planarians will be subjected to RNA sequencing and the results will be compared against the baseline control group to determine the gene expression changes that occur with carcinogen administration.

Once the genes involved in tumor suppression are evaluated through RNA sequencing, RNA interference (RNAi) will be performed to determine whether these genes are required for tumor suppression in planarians. Again, there will be a step-wise increase of carcinogenic administration to determine if the level of carcinogenic material affects the efficiency of these mechanisms. The same four groups and procedures will be used as above except that the genes found to be involved with tumor suppression in the previous results will be silenced in all four groups using RNA interference. Additional control RNAi groups will be utilized to act as a baseline to compare against the candidate gene RNAi and then to compare the extent of tumor formation. Again, *in situ* hybridization and RNA sequencing will be conducted on every planarian to determine at what time point and carcinogen concentration the presence of tumors emerges and the gene expression changes, respectively.

Lastly, the genes found with experimentation to be involved in tumor suppression will be fully sequenced, and online databases of human orthologs will be searched. These human orthologs will now serve as a possible means of formation of future anti-cancer therapies.

EXPECTED OUTCOMES

With the first experiment, healthy, intact planarians that received carcinogens would initially be expected to show signs of tumor suppression in the results. These signs would include little to no abnormal DNA shown with visualization software and an up-regulation of genes involved in tumor suppression in the RNA sequencing of the planarians. Once the tumor suppression system is exhausted and cancerous cells form, abnormal DNA would be quantified with visualization software, but the expression of the tumor-suppressing genes may be either up- or down-regulated. Since cancers affect the DNA of dividing cells, and neoblasts are the only mitotically active cell within a planarian, it can be hypothesized that the distribution of cells with mutated DNA, before the formation of a tumor, will occur through neoblasts. The regenerating planarians administered carcinogens are expected to produce similar results. However, previous research has found a specific tumor-suppressing gene in planarians, *Smed-p53* which is mainly expressed in newly made stem cell progeny (Pearson and Sanchez Alvarado, 2009).

It can be expected that the results would be exaggerated with respect to that of group 2 due to increased neoblast population induced by amputation. It is anticipated that a more concentrated dose of carcinogen would be needed to induce tumor formation; therefore, a longer amount of time would pass in which no abnormal DNA would be seen. Also, the expression of the tumor suppression genes with RNA sequencing is expected to be higher relative to that of healthy, intact planarians in group 2. The irradiated planarians in group 4 are not expected to form tumors due to the absence of proliferative cells. It can be expected that an increase in proliferation would occur in all of the experimental groups with a knockdown of

the tumor-suppressing genes if no other mechanisms exist to ward off hyper-proliferation in planarians. A similar result was observed in an experiment conducted by Sanchez Alvarado (2012) in which RNA interference was used to inhibit activity of the tumor-suppressing gene, *Smed-p53*. *Smedp53* (RNAi) animals are expected to develop tumors not observed in normal control animals. Again, healthy, intact group 1 planarians would be used as a baseline for all of the results obtained from the experimental groups. Knockdown planarians from groups 2 and 3 are expected to show less effective tumor suppression than controls, and are expected to display an increase in abnormal DNA with lower concentrations of administered carcinogen. Again, due to the absence of neoblasts in the irradiated planarians of group 4, they would not be expected to form tumors upon carcinogenic administration.

The final stage of this research involves identification of human orthologs to established planarian tumor-suppressing genes. Previous research in planarians has shown that knock down of PTEN by RNA interference in planarians results in the presence of abnormal cells that behave similarly to cancerous cells in humans (Oviedo and Beane, 2009). The human PTEN homolog was found to be the most commonly mutated gene within cancer patients (Oviedo and Beane, 2009). Therefore it can be expected that more human cancer gene orthologs will be found from this project, but the identity of the genes can only be determined by experimentation.

POTENTIAL PITFALLS/ALTERNATIVE APPROACHES

With the first experiment it is possible that cancerous cells never actually aggregate into a tumor with increasing carcinogen administration. While this result would certainly lead to adjustments to the research, it would not be deleterious. This outcome would simply demonstrate superior effectiveness of the planarian's mechanisms for tumor suppression. The up- or down-regulation of genes would still be obtained from RNA sequencing of planarians. This information would be sufficient for continuation into the second section of the research.

Possible setbacks exist that may affect either the first or second portions of this research. The first setback is the possibility that the carcinogen could be unsuccessfully administered. In other words, the carcinogen is not given the opportunity, or is unable, to be incorporated into the cells. Therefore, tumor growth would never occur due to the lack of damaged DNA, which would then never replicate producing unnecessary cells also possessing damaged DNA. In order to lessen the likelihood of this event occurring, control experiments will be conducted before the start of the actual experimental protocol. The possibility of carcinogen ineffectiveness will be eliminated by testing administration of the carcinogen on a small group of planarians not involved in the experiment. As explained above, these planarians would be expected to form tumors upon carcinogenic being administered. If this group does not produce the expected results, a different method of administration will be used, such as soaking the planarians in the carcinogen.

POTENTIAL OF CANDIDATE AND HOST LABORATORY

This research is to be conducted in the Howard Hughes Medical Institute at the University of Illinois at Urbana-Champaign. This lab frequently conducts research using *S. mediterranea* as a model organism and has all of the resources necessary to effectively perform the mechanisms described in this proposal. The candidate researcher displays advanced knowledge of the biological processes to be utilized in this research. The candidate scientist and supporting research staff are competent and thorough in their roles and work together to continually produce accurate and significant results.

SIGNIFICANCE

Planarians have the unique ability to regenerate and reproduce an incalculable number of times from stem cells that proliferate without tumor formation. The ultimate goal of this proposed research is to determine the existence of human orthologs of the genes responsible for tumor suppression in planarians. If this research is successful, a new avenue of possible anti-cancer therapies could result. For example, if genes susceptible to mutation in humans can be identified, it may be possible to create new therapies that strengthen the molecular make-up against mutations that commonly occur in the cancer. Obviously, more research is needed to determine whether or not a therapy based on this study is a possibility in the clinic. More experimentation would be necessary on vertebrate model systems, such as mice. However, if possible, such therapy (or therapies) could improve the quality of life of millions of individuals. Cancer is a devastating disease that was estimated by the American Cancer Society (2014) to affect 1,665,540 new patients and be responsible for 585,720 deaths just this year. This number could be greatly reduced if the results from this research could be utilized for preventative anti-cancer therapies.

CLOSING

The goal of this research is to utilize planarians as a model for cancer prevention in the human body. By using results from previous research and knowledge of the mechanisms associated with the regenerative capabilities of planarians, genes with specific functions can be identified and isolated. In this case, the target genes are involved in the planarian's ability to suppress tumor formation in the face of frequent hyper-proliferation linked to wound healing and regeneration. In order to accomplish this goal, the planarian *S. mediterranea* will be subjected to administration of carcinogenic material and the resultant gene expression changes will be recorded. In hopes that the genes involved in suppressing tumors in planarians are conserved in humans, they will be compared to human genes using various online databases, such as NCBI and SmedGD. Overall, the results of this research hope to shed some light on new possible anti-cancer therapies for high-risk patients. With the potential of advancement of anti-cancer therapies in humans, the National Institutes of Health (NIH) should take this proposed research into consideration for funding.

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