1. SPECIFIC AIMS: Our preliminary data supports the idea that dioxins and related pollutants cause hepatocellular cancer in the mouse through an aryl hydrocarbon receptor (AHR) dependent mechanism that requires downstream inflammatory events involving the IL1 like cytokines. The overarching objective is to develop a mouse model that can be used to identify biomarkers of dioxin and inflammation induced HCC. With these biomarkers in hand, we will then move to human populations in an effort to develop screens to predict individuals at risk for HCC and ultimately other cancers such as CC.

Aim 1: Develop a mutant mouse model that displays a high incidence of hepatocellular cancers resulting from environmentally induced inflammatory mechanisms. We propose to generate a mouse model where an activated AHR can be regulated using the tetracycline-controlled transactivator (tTA). This model will then be characterized for its capacity to induce inflammation mediated HCC in response to tetracycline manipulation.

Aim 2: Identify biomarkers of HCC risk by identification of genes that are responsive to AHR activation and AHR mediated inflammation in the liver. We propose to identify AHR-regulated transcriptional targets and inflammatory targets that play a role in hepatocellular tumor promotion and that have potential to serve as indicators of risk for HCC as well as early disease.

2. STUDIES AND RESULTS

Aim 1: Develop a mutant mouse model that displays a high incidence of hepatocellular cancers resulting from environmentally induced inflammatory mechanisms.
Activation of the AHR in vivo is challenging in that many of the known agonists are toxic and activate the receptor at many tissue sites. Furthermore, the activity of the agent is impacted by its distribution and metabolism [1]. For compounds like I3C, one is also faced with a complex mixture of condensation products, with multiple potential pharmacological activities including AHR agonism [2-8]. In an attempt to overcome the complexity of systemic administration of AHR agonists, we will develop a mouse model expressing a tissue regulatable constitutively active version of the AHR (caAHR). To this end, we have utilized our knowledge of the AHR domains to develop a version of the AHR that is constitutively active in vitro. Using a previously described construct [9], we have demonstrated that a mutant of AHR lacking the PAS B domain is active in the absence of ligand. However, we have found that the caAHR protein production by the reported construct is very low. Therefore, to enhance the caAHR protein productivity, we have developed a new caAHR expression construct using codon optimization and insertion of β-globin/immunoglobin intron. We found that the optimized caAHR expression plasmid dramatically enhanced the expression level of the caAHR protein and the activity of AHR signaling in various cell lines (Figure 1).

Having created the enhanced expression vector, we created a “Tet-off” CA-AHR expression vector (pTRE-βca-AHR OPT) containing the tTA-regulatory promoter, the codon-optimized ca-AHR cDNA and the β-globin/immunoglobin intron (Figure 2). After transient transfection of the pTRE-βca-AHR OPT vector into Tet-off Advanced HepG2 cells expressing tTA (Clontech), we confirmed that the AHR activity was significantly increased in absence of DOX. The induction of AHR activity was inhibited by treatment of DOX in the transfected HepG2 cells.

Gregory D. Kennedy, MD, PhD
We have finally generated mice harboring the pTet-CA-AHROPT expression cassette (ca-AHRopt) (Figure 2-C). To create both global and tissue-specific ca-AHR expression mouse models, the ca-AHRopt mice were crossed to the conditional tTA expression mice, Gt(Rosa)26Sortm5(ACTB-tTA)Luo/J (Figure 3) (7). Breeding to animals with global or tissue-specific expression of the Cre-transgene, the resultant mice would display global or tissue-specific ca-AHR expression mice (Fig.3). For example, an intercross of tTA/caAHR with CreAlb/+ will generate animals with caAHR expression specifically in the liver. By intake of dox dissolved in drinking water, the caAHR expression in the hepatocytes would be manipulated in these mice.

While we have generated several founder lines of C3H mice carrying this transgene, before we can perform experiments we must generate a congenic C57/Bl6J mouse strain carrying the transgene. We have now performed approximately 6 back-crosses onto the C57/Bl6J strain of mice and have nearly isolated our congenic animal. Once this is complete, we can begin to perform the experiments described.

3. BACKGROUND AND SIGNIFICANCE:
Liver cancer is the fifth most common cancer worldwide with 564,000 cases reported in 2000 [10]. The incidence of this disease will likely continue to increase in developed countries. The major risk factors for HCC are infection with hepatitis C virus, alcohol use, tobacco use, oral contraceptives, nonalcoholic fatty liver disease, and exposure to environmental toxicants. We postulate that all of these risk factors share hepatic inflammation as a common factor. If this idea is correct, then screening for biomarkers of subclinical hepatic inflammation could be used as a screen to identify individuals at high risk for HCC. A successful screen of this type could save thousands of lives annually through inexpensive, routine “inflammation surveillance”.

Gregory D. Kennedy, MD, PhD
The Wisconsin environment is contaminated with a number of persistent chlorinated dioxins and biphenyls (PCBs) that are classified as known or suspected human hepatocarcinogens [11]. Our recent evidence suggests that these pollutants cause liver cancer through an inflammatory mechanism involving the AHR and downstream signaling events requiring cytokines of the IL1/TNF-alpha class. In May of 2001, the Environmental Protection Agency (EPA) determined that dioxin was a “known human carcinogen” and that the risk for individuals most highly exposed is 1 in 1000 [12]. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (“TCDD”), the prototype for this family of pollutants causes HCC in mice and rats. Dioxin has been shown to be a complete carcinogen in a number of animal models. Chronic exposure of rats to dioxin at a level of 2 ppb results in an increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates and tongue [13]. In the Syrian Hamster, a species most resistant to the toxic effects of dioxin, chronic exposure of 600 μg dioxin/kg has been shown to cause squamous cell carcinomas of the facial region [14]. Chronic dioxin carcinogenesis assays using C57BL/6 (B6) hybrids exposed to 30-60 μg/kg resulted in an increase in thymic lymphomas and hepatocellular adenomas and carcinomas [15]. Both susceptible B6C3F1 mice and resistant B6 mice were found to develop liver tumors following chronic administration of dioxin [16]. Dioxin is defined as a complete carcinogen, although TCDD has also been shown to be a tumor promoter in the two-stage model of carcinogenesis in rat liver [17-19] and in the two-stage model in mouse skin [20], lung [21] and liver [21].

Our studies above will ultimately provide a novel animal model to study AHR activation in a tissue specific fashion without any potential carcinogen exposure. This model will prove powerful in a number of future studies.

4. Future Directions

Specific Aim 1

We have had some difficulty ultimately obtaining the transgenic line as described. However, it is nearly congenic on the C57/BL6J background and we expect to begin experiments within the next 3-6 months. This line is going to prove critical to moving our laboratory progress forward. Furthermore, it is only because of the support of the SSAT that we were able to generate this transgenic animal. Furthermore, the support provided by the SSAT at this critical time in our laboratory allowed us to get through a difficult time in our funding until we were able to secure NIH funding.

Specific Aim 2

We have no plans to pursue aim 2 over the next year. We have redirected our laboratory to the field of colon cancer chemoprevention which is closely aligned with the PI’s clinical interest.

5. Publications


8. Kennedy GD, Nukaya M, Moran SS, Glover E, Hecht SS, Drinkwater NR, Pitot HC, Weinberg S, Bradfield CA. Dioxin-induced liver tumor promotion is dependent on binding affinity of aryl hydrocarbon receptor for dioxin and tumor necrosis factor/Interleukin 1 cytokines signaling. (manuscript in submission-Proceedings of the National Academy of Sciences)

6. Project-Generated Resources

None

7. Research Development.

I have participated in weekly lab meetings as well as weekly informal meetings with my mentor. I have attended two key clinical scientific meetings. We have submitted a manuscript examining the role of AHR in hepatocarcinogenesis.

8. Other Activities.

As a practicing surgeon, I have maintained a manageable clinical practice. This includes taking general surgery call 3-4 times/month, ½ day of clinic/week, ½ day of procedures/per week and one elective operative day/week. I spend approximately 25-50% of my time each week on these activities. These activities remain critical to my professional development.

My teaching responsibilities revolve around those students who are in the clinical years of medical school. I have no teaching responsibility outside of the hospital wards and operating rooms. I have just been awarded the Medical Alumni Association 2011 Distinguished Award for Clinical Science Teaching. This is given by the graduating medical students each year to one clinical faculty at the University of Wisconsin School of Medicine and Public Health.

9. Research Development and Other Activities Planned for the Next Year.

Over the last year, I have performed experiments to assess the role of AHR in colon cancer. These experiments have culminated in the submission of a grant to the NIH which has been funded at the level of an R01 (1R01ES020900-01A1). Over the next year, we are going to submit a minimum of two papers from the laboratory investigations we have performed thus far. In addition to writing these manuscripts, we have presented our data at two national meetings. We anticipate presenting at 2-3 national meetings in 2013- 2014.

In addition to developing our independent research program, we continue to actively collaborate with others. We continue to actively collaborate with Dr. Christopher Bradfield. In addition, we have
Role of aryl hydrocarbon receptor and inflammation in GI malignancies

significant contribution to work with others here at the UW and other institutions. These collaborations have resulted in two separate papers with us as co-authors.

All of these activities have led to my promotion to Associate Professor with Tenure at the University of Wisconsin. The SSAT Career Development Award allowed me the freedom to pursue these high risk projects leading to the R01 funding and my final promotion. I plan to continue these experiments in order to fully validate the mouse model and use this in our chemoprevention line of investigation.

10. References

Role of aryl hydrocarbon receptor and inflammation in GI malignancies


