MicroRNAs miR-221 and miR-181c Regulation of Liver Tumor Growth and Metastasis in Mouse Progeny from Dibenzo[*def,p*]chrysene Dosed Mothers Fed a Sulforaphane Diet

Chelsea Meedom[1], Lisbeth K. Siddens[1], Christiane V. Löhr[3], David E. Williams[1,2]

[1]Department of Environmental and Molecular Toxicology, [2]Linus Pauling Institute, and [3]College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331.

Background

Dibenzo*[def,p]*chrysene (DBC) is a polycyclic aromatic hydrocarbon (PAH) and an IARC classified 2A probable human carcinogen. Mouse progeny transplacentally exposed to DBC have been shown to develop lung and liver tumors, with liver tumors developing predominantly in males later in life. Sulforaphane (SFN) is an isothiocyanate that plays a crucial role in cellular protection from carcinogens, such as DBC, and can be found in cruciferous vegetables. MicroRNAs (miRNAs) are a class of small, less than 200bp, non-coding RNAs that regulate gene expression post-transcriptionally. Emerging evidence has shown that miRNAs play critical roles in the development and progression of hepatocellular carcinoma (HCC). miR-221 and miR-222, have been found to promote HCC cell growth by increasing cell numbers during the S-phase of DNA replication [Huang and He, 2011]. miR-181c has been found to be downregulated in primary neuroblastoma (NB) tissues, which implies that miR-181c may be associated with cancer development [Li, Wang, Li, and Yue, 2013]. Pregnant mice were given one dose of DBC (15mg/kg) on gestation day (GD) 17 while fed a control or SFN diet (400ppm) starting on GD 9 and continued through weaning. Weaned offspring were maintained for 10 months and tissues analyzed for tumors. In this experiment, it was found that miR-181c was significantly up-regulated in tumors from both treatment groups. miR-221 was found to be significantly upregulated in the DBC/SFN treatment group. Proteins that are targeted by miR-181c have been shown to inactivate mechanisms which oncongenes use to metastasize in tissues. [Chen, Wang, Xu, Guo, Jiang, 2015] There is growing evidence that MicroRNAs are critical components in cancer progression and initiation. miR-221 has been identified as a regulator of epithelial-to-mesenchymal transitions [Shah and Calin, 2011]. miR-181c has been shown to suppress tumor necrosis factor in microglial mediated neuronal apoptosis [Zhang et al., 2012]. The upregulation of miR-181c and miR-221 suggests these miRNAs may be involved in hepatic cancer development in this model.

Methods

- See "Experimental Design" for a description on the mouse model experiment where livers were harvested and archived.
- Formalin fixed livers were analyzed by using calipers to measure the excised tumors diameters. Select tumors were blocked, mounted, and H&E stained then characterized with microscopy.
- TRIzol™ reagent was used for RNA extraction from frozen tumor and control liver tissues. RNA quality was determined with an Agilent TapeStation 4200 (RIN= 9.13 ±0.3). A Qiagen miScript II RT Kit (#218160) was used to synthesize cDNA and measure miRNA levels using qPCR on a BioRad iQ5 Thermocycler.
- Bioinformatics Resource Manager (BRM) Software was used to predict target genes for tissue specific miRNA. http://www.cbb.pnl.gov/brm

Results

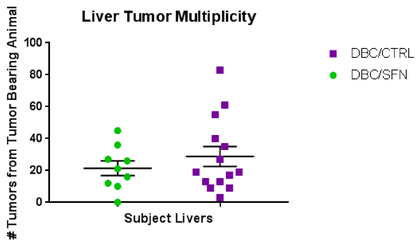

Figure 1a. Tumor multiplicity for test subject livers in DBC/CTRL and DBC/SFN dietary groups.

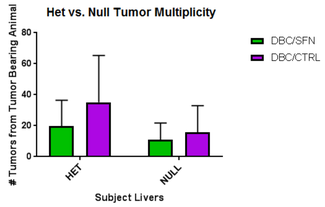

Figure 1b. Tumor Multiplicity for test subject livers separated by *Nrf2* heterozygous vs *Nrf2* null

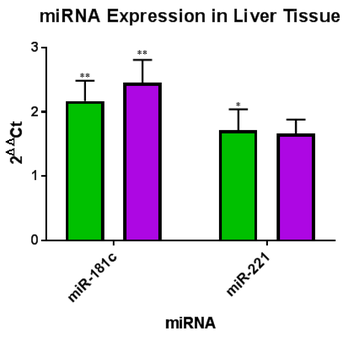

Figure 2. miRNA expression of liver tumors relative to control tissue calculated using ΔΔCt method and Snord-72 as a reference non-coding RNA. ** indicates p<0.01, * indicates p<0.05

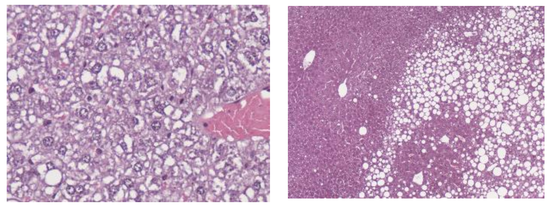

Figure 3. Histological images of liver tumors.
Left: Subject liver exhibiting hepatocellular carcinoma arising from adenoma. Note the extensive necrosis.
Right: Subject liver exhibiting hepatocellular hyperplasia focusing on the vacuoles of the liver.

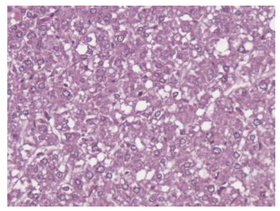

Figure 4.Histological image of liver tumors. Subject liver exhibiting hepatocellular adenoma, clear cell type.

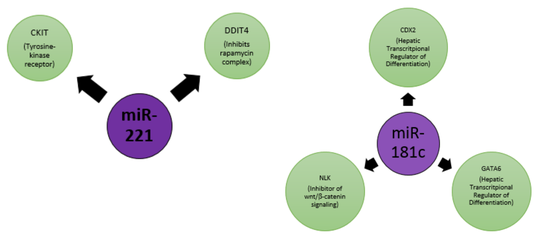

Figure 5. Predicted targeted proteins for miR-181c and miR-221 found using BRM Gene Prediction Software.
Left: miR-221 upregulates CKIT & DDIT4. DDIT4 is a bona fide proto-oncogene known to inhibit the rapamycin complex.
Right: miR-181c upregulates CDX2, GATA6, and NLK. CDX2 and GATA6 are regulators of differentiation for hepatic transcription. NLK is an inhibitor of wnt/β-catenin signaling.

Experimental Design

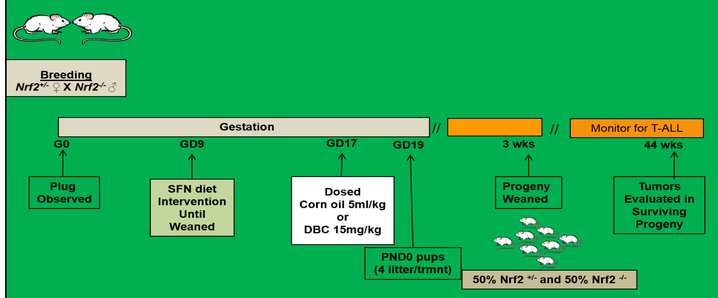

Figure 6. 4 treatment groups: Corn oil control diet/DBC control diet/corn oil with SFN diet/DBC with SFN diet.
Mice were sacrificed if any signs of morbidity or pain were exhibited.

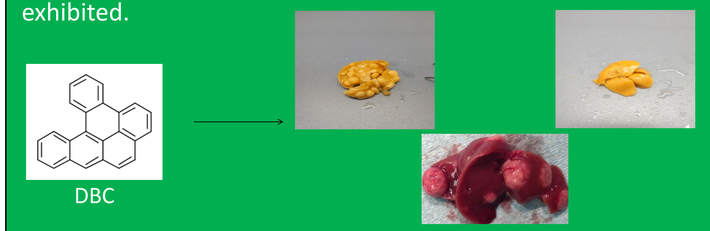

Figure 7. Progeny mouse livers treated with DBC transplacentally. Left: fixed het liver. Center: fresh het liver. Right: fixed null liver.

Conclusions & Future Work

- miR-181c was significantly (P<0.01) upregulated in both treatment groups (Figure 2)
- miR-221 was upregulated in both treatment groups with the DBC/SFN diet group being significant (P<0.05). (Figure 2)
- Sulforaphane dietary intervention did not reduce or increase regulation significantly when compared to the referencing control liver tissue
- miR-221 is predicted to target proteins DDIT4 and CKIT . (Figure 5)
- miRNA levels were not affected by loss of *Nrf2* in null groups compared to heterozygotes. (data not shown)
- Further analysis of gene targets for miR-181c and miR-221 will be performed.

Works Cited:
1. *Huang et al. 2011. British Journal of Cancer 104 (2), 235-240*
2. *Li et al. 2013. Acta Biochimica et Biophysica Sinica 46(1)*
3. *Chen et al. 2015. Oncotarget. 42:44466-44479*

Contact Information:
Chelsea Meedom
meedomc@oregonstate.edu