Evaluating canine aryl hydrocarbon receptor's role in osteosarcoma progression, metastasis, and chemoresistance

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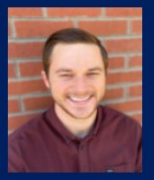

BACKGROUND

The aryl hydrocarbon receptor (AHR) is a cytosolic transcription factor that binds to specific DNA segments and alters the activity of nearby genes. These DNA sequences are dispersed in the genomes of many species and allow AHR to interact with thousands of different genes. It has been shown that AHR plays a role in the regulation of numerous biological processes such as cell signaling, cell cycle, metabolism, and much more[1]. AHR can be activated by a large number of ligands. Some AHR ligands, such as dioxin or TCDD, are environmental toxins and carcinogens, while others appear to contribute to normal biological function and even show anti-tumor effects. For

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HYPOTHESIS AND AIMS

Hypothesis: Activation of the canine AHR inhibits osteosarcoma (OSA) growth and migration by regulating oncogenes and tumor suppressor genes and contributes to the cytotoxic effects of chemotherapy

AIM 1. Determine the effect of AHR silencing on chemotherapy resistance in canine osteosarcoma cell lines.

AIM 2. Evaluate the effects of AHR activating ligands Omeprazole and I3C on canine tumor migration.

AIM 3. Identify genes regulated by canine AHR that are involved in tumor progression and metastasis.

METHODS

AIM 1: Proliferation Assay – one of three OSA cell lines (Abrams, Gracie, and HMPOS) were seeded into 96-well plates. As seen in the Western Blot below, these cell lines were chosen because Abrams and Gracie have been shown to exhibit high levels of AHR expression while HMPOS has relatively low AHR protein expression. Once seeded in 96-well plates, cells were treated with 1) 25nM AHR small interfering RNA (siRNA) which targets and knocks down AHR expression, 2) 25nM scramble, aka control, siRNA that should target nothing, or 3) siRNA vehicle, in this case Opti-MEM media. After exposure to siRNA or vehicle for 24 hours, the wells were washed and treated for another 24 hours with 1) 200nM standard chemotherapy agent Doxorubicin, 2) 200μM standard chemotherapy agent Carboplatin, or 3) drug vehicle, in this case just water. In the last 24 hours, the cells were incubated with only media and no treatment. Alamarblue was added 2 hours prior to fluorescence reading on a Synergy H1 microplate reader.

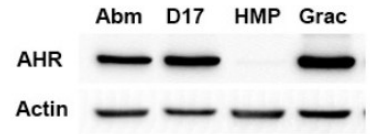

Figure 1: Western blot performed on a variety of OSA cell lines (Abm = Abrams, HMP = HMPOS, Grac = Gracie). HMPOS exhibits low AHR protein expression when compared to Abrams and Gracie.

AIM 2: Scratch Assay – one of four cell lines (Abrams, Gracie, HMPOS, and MDCK) were seeded into 48-well plates. MDCK was included in

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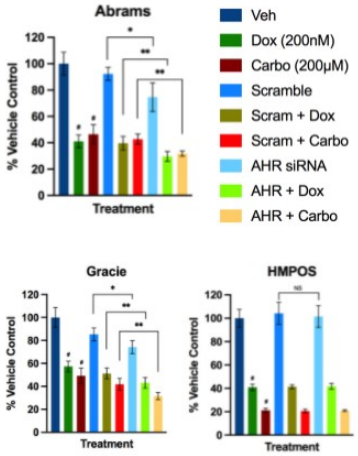
RESULTS

AIM 1:



As expected, all 3 cell lines showed a significant decrease in proliferation when treated with either doxorubicin or carboplatin. For the Abrams and Gracie cell lines, there was a significant decrease in proliferation between the scramble siRNA and AHR siRNA groups. This data supports the hypothesis that AHR plays a pivotal role in OSA proliferation. Concurrently, a significant decrease also occurred between scramble siRNA + doxorubicin or carboplatin groups and the AHR siRNA + doxorubicin or carboplatin groups respectively. This evidence hints towards AHR

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CONCLUSIONS

The data collected from AIM 1 supports the idea that AHR plays an important role in canine OSA proliferation. This was concluded due to the significant decrease in OSA proliferation when AHR expression was knocked down using AHR siRNA. Future studies should aim to identify what would happen to OSA proliferation when AHR is activated through a variety of ligands rather than knocked down. Further data from AIM 1 identifies AHR as playing a potential role in OSA chemotherapy resistance. The supporting evidence was a significant decrease in OSA proliferation when treated with AHR siRNA in combination with chemotherapy agents versus control siRNA in combination with chemotherapy agents. RNAseq data that is currently being processed should provide intel on if the data is AHR-specific or due to more complex interactions when doxorubicin

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References

1. Kawajiri K, Fujii-Kuriyama Y. The aryl hydrocarbon receptor: a multifunctional chemical sensor for host

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