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The Role of the Aryl Hydrocarbon Receptor in Tumor Growth and Chemoresistance

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The Role of the Aryl Hydrocarbon Receptor in Tumor Growth and Chemoresistance.

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The aryl hydrocarbon receptor (AHR) has been shown to play a role in cancer initiation and progression in oral squamous cell carcinomas (OSCC), and other cancers. The AHR is activated by environmental toxins, including polycyclic aromatic hydrocarbons, which are commonly found in cigarette smoke. It is hypothesized that activation of the AHR by these environmental toxins can contribute to the growth and chemoresistance of OSCCs. Nude mice tongues were injected with a human OSCCs cell line, SCC2s, and treated with an AHR antagonist at 25mg/kg daily via oral gavage. Primary tumor growth was measured via calipers and IVIS imaging. RT-qPCR analysis of the harvested tongue tumors and livers was used to examine the activity of the AHR by quantifying the expression levels of *Cyp1b1* and *Cyp1a1*. Based on the results of the *in vivo* experiments, continued testing was conducted to examine the role of AHR inhibition in chemoresistance. Using MTT cell viability assays coupled with dosing of commonly used chemotherapeutics, the effects of the AHR on the chemo-resistance of SCC2s was tested. Three commonly used chemotherapeutics were tested at various dose ranges: Cisplatin (0-10uM), doxorubicin (0-1uM), and 5-Fluorouracil (0-10uM). In addition, cells were co-treated with an AHR antagonist (5uM CH223191) and the chemotherapeutic to determine if decreasing AHR activity increased chemotherapeutic efficiency. ANOVAs were used to evaluate the significance of AHR activity on the effectiveness of the chemotherapeutics. It was determined that AHR antagonism with CB7993113 significantly affected OSCC primary tumor growth *in vivo*. Additionally, it was found that both *Cyp1a1* and *Cyp1b1* expression decreased after treatment with CB7993113 when compared to vehicle alone in the tongue. In the liver, it was found that both *Cyp1a1* and *Cyp1b1* expression also decreased after treatment with CB7993113 when compared to vehicle alone. Interestingly, we also found that decreasing AHR activity with an AHR antagonist CH223191 in addition to treatment with a chemotherapeutic lead to a significant increase in cell death when compared to treatment with the chemotherapeutic alone. This phenomenon was observed in three different frontline OSCC therapeutics. These novel findings implicate the AHR in OSCC initiation and growth, also supporting the development of AHR modulators as potential chemotherapeutics. Overall, these findings support the hypothesis that the activation of the AHR is linked to tumor growth of oral squamous cell carcinomas as well as contributing to the potential chemoresistance of these cells.

Introduction:

Oral cancer affects approximately 50,000 individuals per year, demonstrating a kill rate of one individual per day and is a cancer that kills more than many other well-known cancers. Oral cancers are defined as cancers of the oral cavity, throat, and pharynx. Most often diagnosed in their later stages, oral cancers are difficult to effectively treat due to their concealed nature in the oral cavity. Also complicating the treatment process, there is extensive variation in the kinds of oral cancers, with varying causes. The most prevalent factors in oral cancer development are race, age and carcinogen use. Comprehensive studies reveal that oral cancers are twice as prevalent in men as women, but more recent studies indicate a slow rise in oral cancer occurrences in women. The same study also demonstrates a higher occurrence of oral cancers in black men over white men. Both of these trends are attributed to early statistic indicating a higher usage of tobacco products in men over women and more specifically in black men. In the early 1900's tobacco usage was much more prevalent among men over women. In the last few decades, the occurrence of tobacco use in women has increased, thus contributing to the increased prevalence of oral cancers among women. Age also places individuals at high risk for the occurrence of oral cancers. Most oral cancers are incredibly slow growing, and emerge after prolonged use or exposure to a certain compound. These factors contribute to the occurrence of oral cancers in the later stages of life, the average age of diagnosis being over 40. But arguably the biggest impact on the occurrence of cancer is prolonged exposure to potential carcinogens. Some of these carcinogens could be UV radiation, HPV infection in the mouth, and most prevalent is the use of tobacco products such as chewing tobacco and cigarettes. In the U.S tobacco

use resulted in 30% of all deaths, the majority cancer related. While it is very evident what can increase the risk of oral cancer within and individual, there is little known information about what actually causes the cancerous cells. While there have been a number of connections and discovered, there are still many avenues to discover. One of the largest questions in the field of cancer research is asking how can tumors and cancer cells metastasize, and why does this occur. One of the possible answers for this is the aryl hydrocarbon receptor.

The aryl hydrocarbon receptor (AHR) is a mammalian protein found in a variety of cells. The AHR acts as a transcription factor within the cells, most notably regulating xenobiotic metabolic enzyme such as cytochrome P450 by control transcription of the genes *Cyp1a1* and *Cyp1b1*. As a AHR ligand binds the protein, the AHR will dimerize with the AHR nuclear translocator (ARNT), translocate to the nucleus, and begin transcribing the DNA. The AHR is considered a helix-loop-helix transcription factor and can bind a number or native or foreign ligands. Within the body, the AHR can bind flavonoids and indoles among other naturally occurring AHR ligands. But, the AHR can also favorably bind polycyclic aromatic hydrocarbons (PAH) or dioxin/ dioxin-like compounds [1]. These non-native ligands are commonly ingested and inhaled due to their elevated levels in water, food and air. PAHs are present in burnt food material, such as grilled meat, wood fired pizza and a number of other sources. Dioxin and similar compounds are byproducts of combustion engines and manufacturing plants among other things. Most importantly both of these non-native ligands are common byproducts of chewing tobacco and cigarettes. The activation of the AHR by these non-native ligands is the link between oral cancer and the AHR [1,2].

Activation of the AHR by PAHs or dioxin can induce over expression of the regulated genes *Cyp1a1* and *Cyp1b1*. Research has shown that differential or overexpression of these genes can result in elevated tumor growth as well as increased potential for tumor metastasis in a variety of cancers including breast and oral cancers. The increased of cytochrome p450 can result in procarcinogen production within tissues, but the exact mechanism of this production is not yet known. [2] What is currently understood is that inhibiting the ability of the AHR to transcribe can impact the expression levels of the HAR target genes. This potentially reduces the negative effects stemming from overexpression of the cyp1 genes. Currently there is one well established AHR inhibitor, 2-methyl-2*H*-pyrazole-3-carboxylic acid (2-methyl-4-*o*-tolylazo-phenyl)-amide (CH223191). [3] CH223191 inhibits the AHR by preferentially blocking the binding of AHR ligands as well as inhibiting translocation into the nucleus. It has also shown to modify the DNA binding domain of the AHR, blocking transcription. But, this inhibitor has demonstrated a number of cytotoxic effects due to little knowledge of the mechanisms guiding this inhibition. Among other things, the inhibitor's cytotoxic effects range from an altered differentiation pattern of Th17 cells to the development of severe autoimmune encephalomyelitis. [3] Because of these cytotoxic effects, the search for a less toxic and still effective AHR inhibitor continues as an aim of current research within the field.

Not only does inhibiting the AHR directly influence potential treatment of cancer cell, but it appears that inhibition of the AHR can contribute to efficiency of other treatment as well. Preliminary research with stem-like breast cancer cells indicated that inhibition of the AHR could contribute to an increased sensitivity to chemotherapeutic

treatment [1,4]. This preliminary data calls for an increased understanding as to why this inhibition occurs and through what mechanism. Also, the preliminary data must be confirmed for a number of different chemotherapeutics in order to replicate these early findings as well as understand if AHR is a coupled or independent process from chemotherapeutic treatment. Cytochrome P450, which is regulated by the AHR, has shown to pay a number of roles in both cancerous and non-cancerous cells. But one role in particular is notable. A recent study has indicated that the over expression of cytochrome P450 due to AHR over-activation may contribute to the inhibition of pharmaceuticals but increase the rate at which the cancerous cells can remove the chemotherapeutic. [4] Because of this, coupling AHR inhibition with chemotherapeutic treatment becomes a logical idea, barring that the combination therapy has few cytotoxic effects.

Today there a number of chemotherapeutics that have emerged as some of the best treatments of oral cancer. Doxorubicin, Cisplatin and 5-Fluroruracil, shown below, are at the top of the treatment list, often given as individual therapies in place of combination treatments. Doxorubicin is a leading treatment for a number of cancers, including breast and oral cancer. Doxorubicin is an intercalating agent, which is used to block DNA transcription in cancerous cells by preventing the binding of topoisomerase II. Doxorubicin can also induce histone eviction from actively replicating cells. This deregulates the cell's DNA damage responses and epigenetic responses initiating a cell death response [5]. Along with doxorubicin, cisplatin is another treatment at the forefront of the battle against cancer. Cisplatin is in the class of platinum containing cancer drugs, a newer variety of treatments. The platinum complexes preferentially bind guanines in

DNA strands, to multiple locations on the platinum complex as the chloride substituents are replaces by guanine. This binding of DNA stands results in a crosslinking of strands, blocking DNA replication and inhibiting mitosis. This DNA crosslinking also elicits the DNA damage repair response initiating apoptosis of cells with the crosslinked DNA [6]. 5-flurouracil is another DNA targeting chemotherapeutic, but instead of directly targeting DNA it targets the factor required for replication. It is known as a suicide inhibitor, and targets thymidylate synthase, blocking the production of thymidine, a nucleoside required for DNA replication. The 5-flurouracil creates a deficiency in dTMP resulting in thymine less apoptosis in rapidly dividing cells [7]. Each of these chemotherapeutics is effective at killing cancerous cells, but many have systemic cytotoxic effects at their dosages. A number of cell types have exhibited resistance to the chemotherapeutics and it is hypothesized that this is due to the cells ability to evacuate the drugs from the interior of the cells, preventing cell death. If this is the mechanism by which these cells are able to avoid death by the chemotherapeutic, the hypothesis that AHR inhibition will decrease the chemo resistance of cells stands.
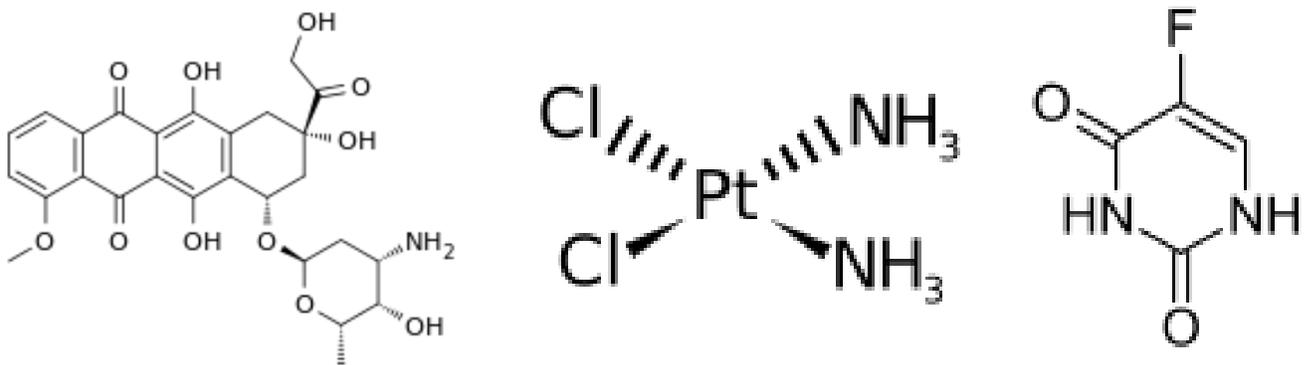

Image 1: The above image shows the structures of three leading chemotherapeutics currently used to treat a number of cancers including breast and oral cancer. The structures above are doxorubicin (left), cisplatin (middle) and 5-flurouracil (right)

objective is to generate an activity profile for two lab-generated AHR targeting

compounds and determine whether these compounds are actively targeting the AHR and if they are acting as an AHR agonist or antagonist. The second objective is to determine how AHR impacts oral cancer cell response to the chemotherapeutics doxorubicin, cisplatin and 5-flurouracil. Each of these objectives will contribute to a greater understanding of the role the aryl hydrocarbon receptor plays in cancer development, and will impact the lives of many suffers with its contributions.

Materials and Methods:

Tissue preparation qRT-PCR analysis:

Immune compromised mice were injected at 6weeks with oral squamous carcinoma cells directly into the tongue. Once tumor growth was detectable in the tongue, oral lavage of Sesame oil, the compound CB7993113 or the compound CB7950998 was given daily at 25mg/kg. After tumors reached their maximum growth limit mice were sacrificed. From the mice, the primary tumor tissue was extracted as well as the liver. RNA from the tissue samples was extracted using the quiagen RNA extraction kit and concentrated for qRT-PCR.

qRT-PCR analysis

qRT-PCR results of the tissue extracted RNA were analyzed to determine expression of the genes *Cyp1a1* and *Cyp1b1*. For the primary tumor data, the samples cells were normalized to OSCC2 cells grown in culture. For the liver tissue, samples were normalized to the oil treated control mouse tissue. Normalized treatment groups were then averages and the differing treatment groups were compared to the control treatment group.

MTT assay:

The MTT assay for cell viability was conducted with OSCC2 cells. These cells were plated in 96 well titre plates at 3500 cells/well. The cells were grown with plain media to ~50% confluency. At 50% confluency, cells were dosed with the desired chemotherapeutics. For the doxorubicin control treatment group, cells were dosed with 0, 0.01,0.1,1.0 and 2.0µM doxorubicin. For the experimental group, the same dosages were repeated with the addition of 5µM CH223191. For the cisplatin control treatment group, cells were dosed with 0, 0.1, 0.5, 1.0 and 10.0µM cisplatin. For the experimental group, the same dosages were repeated with the addition of 5µM CH223191. For the 5-flurouracil control treatment group, cells were dosed with 0, 0.01,0.1,1.0, 10.0, 50.0 and 100.0µM 5-flurouracil. For the experimental group, the same dosages were repeated with the addition of 5µM CH223191. The treated cells were then grown for an additional 24 hours. Following the 24-hour treatment the standard MTT assay protocol was followed to measure cell viability of the OSCC2 cells.

Results:

qRT-PCR Analysis of Tissue Samples

Analysis of primary tissue samples from murine models were conducted using qRT-PCR to determine the expression levels of AHR target genes *Cyp1a1* and *Cyp1b1*. This analysis was critical to determine the effectiveness of two lab generated, active bindingsite analogs, AHR targeting compounds expected to act as an AHR antagonist (CB7993113) and an AHR agonist, CB7950998. qRT-PCR was conducted on RNA extracted from the tongue containing the primary tumor and the liver. Liver tissue samples were utilized to determine if basal transcription od the *Cyp* genes by the AHR

was effected by the AHR targeting compounds, or if only the site of the primary tumor was effected during treatment.

Initally tissues samples of the liver were analized to determine the effects of the AHR agonist and Antagonist on basal transcription of both *Cyp1a1* and *Cyp1b1*. For the gene cyp1a1, the suspected AHR antagonist, CB7993113 actively decreased basal expression of the gene to a fold change of 1.91 comparatively to a fold change of 35.72 as seen in the control treated mice (Figure 1a). The suspected AHR agonist, CB7950998, also showed decreased basal expression levels in the liver tissue. The CB0998 treated tissues decresed the *Cyp1a1* expression to a fold change of 1.62 compared to the control fold change of 35.72 (Figure 1a). This suspected AHR agonist decreased the fold change for the gene by 0.26 more than the suspecected antagonist. For the liver tissues, the qRT-PCR results also examened the

basal expression levels of the gene *Cyp1b1*. The CB7993113 treatment resulted in a decrease of the basal expression levels of the gene to a fold change of 2.61 compared to the control treatment levels with a fold change of 11.32 (Figure 1b). The CB7950998 decreased the fold change for cyp1b1 expression to a level of 4.37 compared to the control fold change of 11.32 (Figure 1b).

Tissue samples from the primary tumor site were analized with the qRT-PCR in order to determine the effectiveness of the suspected AHR antagonist and suspected agonist to effectivly target the AHR at the tumor site. Analysis of the expression of the gene cyp1a1 in the primary tumor site revealed that the suspected AHR antagonist, CB7993113 effectively decreased the expression to a fold change of .09 compared to the expression in control treated tissues with a foldchanges of 0.25 (Figure 1c). The CB7950998 resulted in

a decreased expression of the cyp1a1 gene to a fold change of 0.12 compared to the fold change of the control tissues at 0.25 (Figure 1c). Analysis of the expression of the gene cyp1b1 was also conducted on the primary tumor samples. CB7993113 treated tissues showed decreased expression of the gene cyp1b1, with a fold change of 0.26 to the control fold change of 0.75 (Figure 1d). The CB7950998 also showed a decreased expression of the gene with a fold change of 0.21 compared to the control with a fold change of 0.75 (Figure 1d). As seen earlier, the suspected AHR agonist resulted in a decrease in expression 0.05 more than the suspected AHR antagonist.
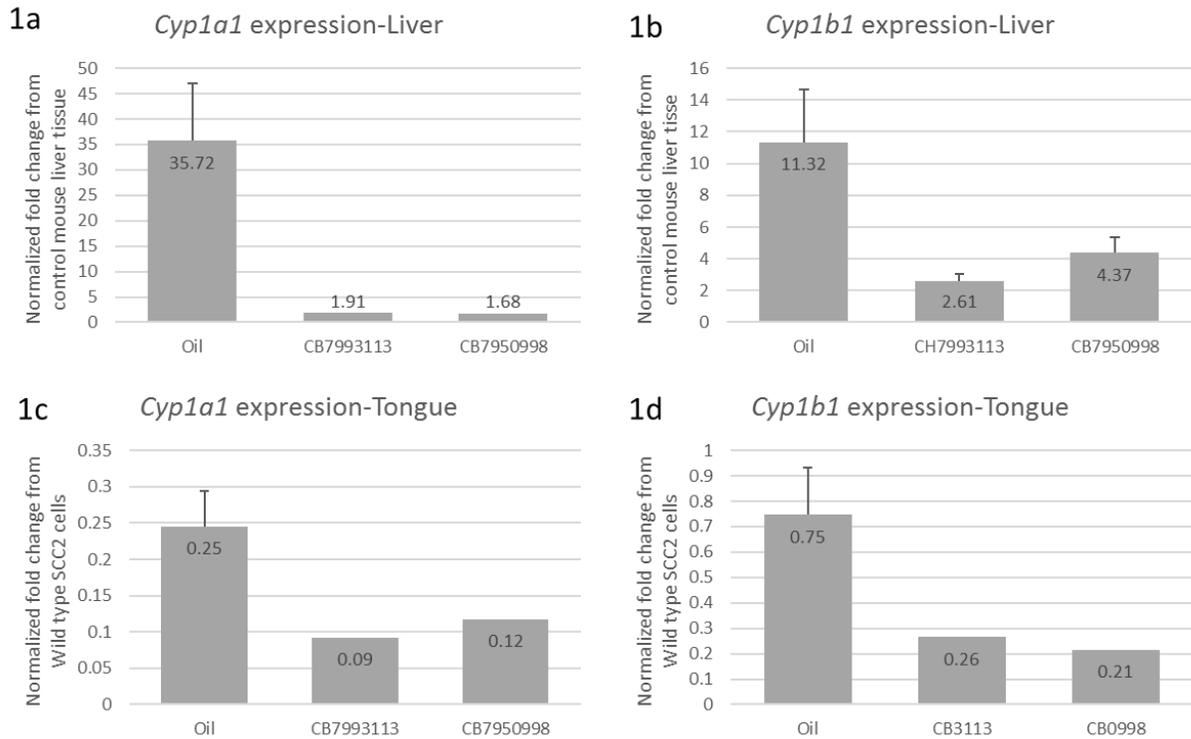

Figure 1: Figure 1a reveals the expression levels of the gene cyp1a1 in liver tissue following oral treatment with 5μM CB7950998 and 5μM CB7993113. Figure 1b shows the expression of the gene cyp1b1 following oral treatment with 5μM CB7950998 and 5μM CB7993113. Figure 1c demonstrates the expression of the gene cyp1a1 in mouse liver tissue following oral treatment with 5μM CB7950998 and 5μM CB7993113. Figure 1d illustrates the expression levels of the gene cyp1b1 in mouse tongue tissue following oral treatment with 5μM CB7950998 and 5μM CB7993113. Expression levels for each sample were obtained using qRT-PCR with RNA extracted from each tissue of the mouse.

MTT Cell Viability Assay

Analysis of the chemosesativity of Oral squamous carcinoma cells was consducted using the MTT assay to determine cell viability of the OSCC2 cell line following chemotherapeutic treatment. Cells were treated with an established dosage range of chemotherapeutic at ~50% confluency. For the control group, only the chemotherapeutic was used. For the experimental group, OSCC2 cells were co-treated with 5µM of the AHR inhibitor CH223191. The MTT assay was conducted following 24Hrs of treatment. OSCC2 treatement with Doxorubicin (doxo) was conducted over the dosage range of 0.0µM to 2.5µM as previously defined in the literature. At 0.0µM doxo the control showed 100% cell viability. The AHR inhibited treatment group also show 100% viability at 0.0 µM Doxo. At doxorubicin concentration increases both treatment groups show a decrease in cell viability to ~50% at 1µM doxo. At 2.5µM doxo, both treatment groups show less than 30% viability, suggesting successful killing of the OSCC2 cells by the chemotherapeutic. Between treatment groups there is not statistical significance in
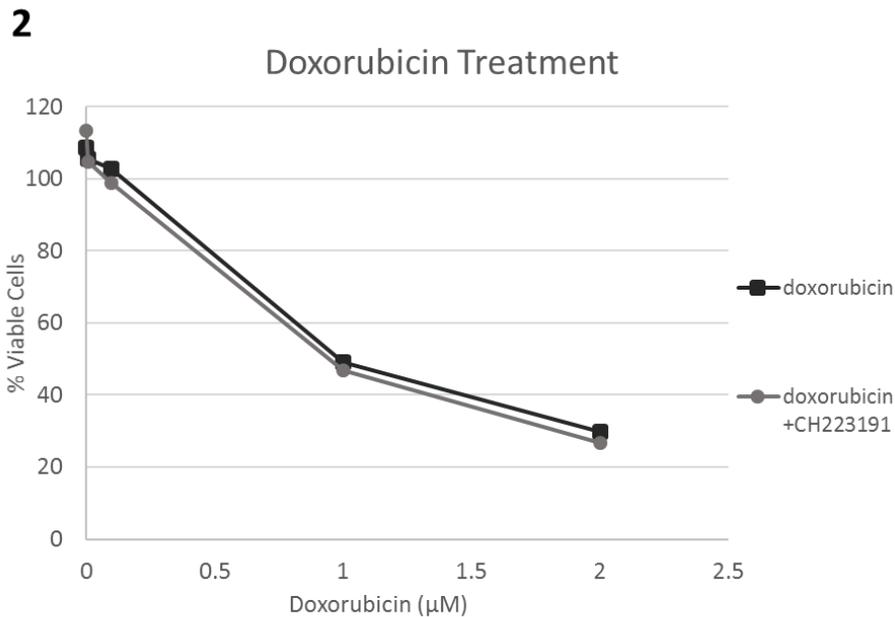

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Doxorubicin Treatment

Figure 2: Figure 2 demonstrates cell viability or oral squamous cells following a 0.0-2.0µM treatment with the chemotherapeutic Doxorubicin. The figure examines the difference between the doxorubicin only treatment group and the doxorubicin+ AHR inhibitor. Statistical significance of 0.005 is indicated.

cell vialbiltity. (Figure 2.)

OSCC2 treatment with the chemotherapeutic Cisplatin (Cplat) was conducted at a dosage range of 0.0 to 10µM. At a concentration of 0.0µM Cplat, cells in the control as well as the AHR inhibited group showed 100% viability. Between the concentrations .5-2.0µM Cplat, both treatment groups show >80% cell viability. Cisplatin at a concentration of 10.0uM showed a cell viability of ~100% for the control group. For the AHR inhibited group, the cell viability at 10.0µM Cplat was ~80%. Neither treatment group was shown to effectively decrease cell viability to below 80% suggesting a number of conclusions.(Figure 3)
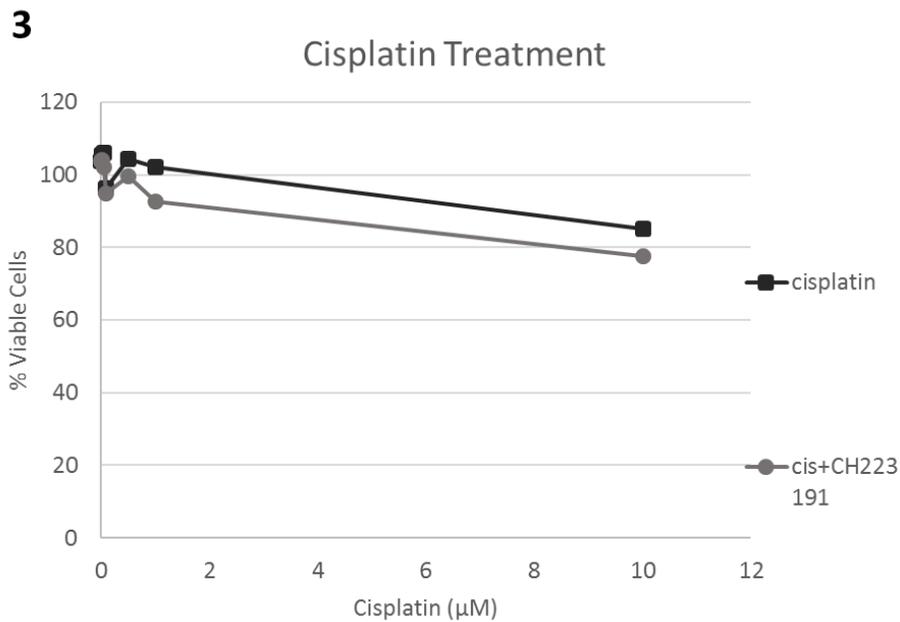
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Figure 3: Figure 3 demonstrates cell viability or oral squamous cells following a 0.0-10.0µM treatment with the chemotherapeutic Cisplatin. The figure examines the difference between the cisplatin only treatment group and the cisplatin + AHR inhibitor group. Statistical significance of 0.005 is indicated.

OSCC2 treatment with the chemotherapeutic 5-Flurouracil (5-FU) was conducted at a dosage range of 0.0µM to 100.0µM. At a concentration 0.0µM 5-FU both the control

treated group and the AHR inhbited group has 100% cell viability following 24 hours of

treatment. At 0.1μM 5-FU the control treated group demontrates a cell viability of ~

100% (Figure 4a). The AHR inhibited group shows a cell viability of ~90%. As the 5-FU

concentration increases to 10.0μM, the control group cell viability drops to 70%. The

Viability of cells in the AHR inhibited group drops to 60% at 10.0μM 5-FU. At a

concentration of 100.0μM 5-FU, cell viability of the control group has dropped to ~50%

or less, suggesting an effectve killing of the OSCC2 cells by the chemotherapeutic. The

AHR inhibited group has cell viability of 40% or less, also suggesting effective killing of

cells in the treatment group (Figure 4b). For the AHR inhibited treatment group, the

effectivness of the 5-Flurouracil was increase significantly at the concentrations of

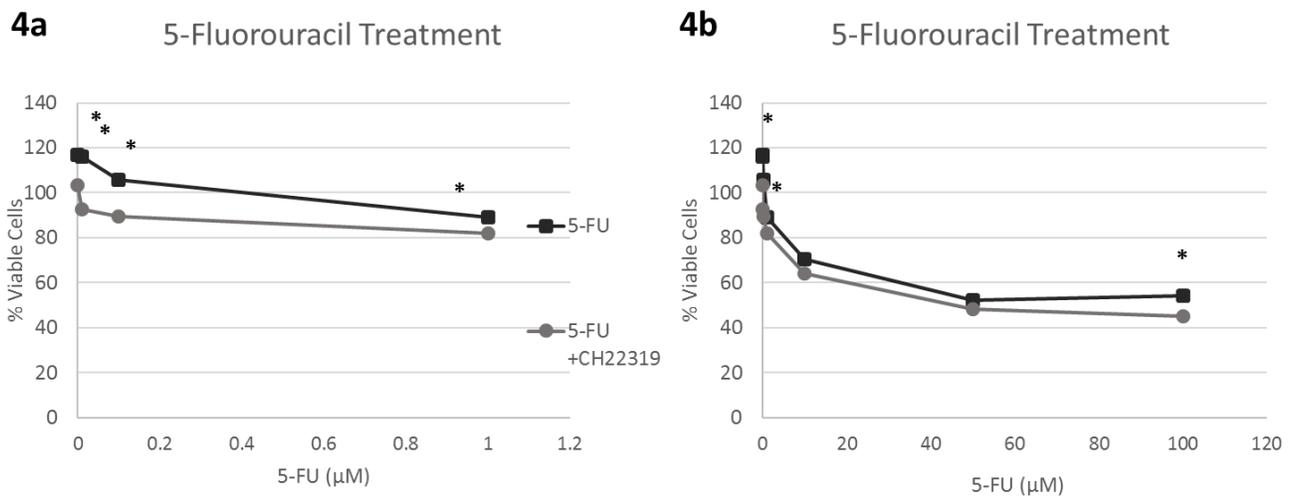0.01,0.1, 1.0, and 100.0μM with the 10.0μM and 50.0μM concentrations trending toward

significance.

Figure 4: Figure 4b demonstrates cell viability or oral squamous cells following a 0.0-100.0μM treatment with the chemotherapeutic 5-flurouracil. Figure 4a offers a closer view of the 0.0-1.0μM region in order to examine the significance between treatment groups. The figure examines the difference between the 5-FU only treatment group and the 5-FU + AHR inhibitor group. Statistical significance of 0.005 is indicated.

Discussion

Suspected AHR targeting compounds reduce expression of AHR target genes

To determine the ability of the two lab generated compounds, CB7993113 and CB7950998, to effectively target the aryl hydrocarbon receptor, the expression of the AHR target genes *Cyp1a1* and *Cyp1b1* was measured through qRT-PCR. For the control group for each of the gene expression tests suggests that there was active AHR transcribing the target genes. Expression levels of the target genes were high in both the primary tumor site as well as the liver tissue. Expression of *Cyp1a1* was higher overall in both tissue samples than the expression of *Cyp1b1* suggesting it is a more favorable target for the AHR over *Cyp1b1*, although this is not a conclusive observation.

CB7993113 was predicted to be an AHR antagonist. If it was an AHR antagonist, there would be notable decreases in the fold change for each target gene, indicating that the AHR was actively inhibited by the CB7993113. This trend was observed in the data. For both the *Cyp1a1* and *Cyp1b1* there was a notable decrease in fold change between the control treated group and the CB7993113 treated group. For both the primary tumor tissue and the liver tissue there was a notable decrease in expression of the genes. The liver tissue decrease is notable important as it indicates that the orally introduced treatment could enter the blood stream and act systemically to inhibit basal levels of AHR activity as well as inhibit activity at the tumor site. Further analysis would need to be conducted to determine the difference expression between directly treated tissues and systemically treated tissues. Because of the significant decreases in the AHR target gene expression in the presence of CB7993113, it can be concluded that this AHR targeting drug is acting as an AHR antagonist.

CB7950889 was expected to behave as an AHR agonist, resulting in increased levels of AHR target gene expression when compared to the control group. But, the trend in the

data suggested the opposite. In most of the tests, the suspected agonist resulted in inhibition if the AHR, showing a decrease in the expression of the AHR target genes. This effect was mirrored in both the primary tumor tissue and the liver tissue, solidifying that the observations made we not due to a secondary influence in the tumor site. This arises the question of why the suspected activator of the AHR instead inhibits the activity. Based on the binding models as well as preliminary in vitro assays conducted, the CB7950998 appeared to boost AHR activity and did not negatively affect cell growth. Thus, the conclusion before the in vivo tests was that the CB7950998 appeared to behave as an AHR agonist. Following the in vivo tests, there is no conclusive evidence that the compound CB7950998 behaves as exclusively and AHR agonist or AHR antagonist. The compound would require further experimentation to uncover in what circumstances the CB7950998 acts as an agonist or antagonist and to provide further insight into the mechanism by which this compound acts on the AHR.

AHR inhibition increases the chemosensativity of OSCC2 cells in select treatment conditions.

The direct mechanism by which the AHR contributes to oral cancer cell chemosensativity is not known, but early studies have revealed that there is a distinguishable correlation between inhibition of the AHR and the chemosensativity of cells. To better understand how this contribution to chemosensativity may be related, a series of cell treatments with chemotherapeutics as well as chemotherapeutics plus the AHR inhibitor CH223191 were conducted. The chemotherapeutics tested in these trails were Doxorubicin, Cisplatin, and 5-Flurouacil. For each chemotherapeutic cell viability was measured for the treatment group with just the chemotherapeutic as well as the

treatment group with chemotherapeutic and AHR inhibitor. The two treatment groups were then analyzed for statistical significance.

Dosage ranges for the chemotherapeutic Doxorubicin was 0-2.0µM, with the AHR inhibited treatment group additionally receiving 5µM of CH22391. Through the conducted trials, there was a lack of statistical significance between the treatment groups. Inhibiting the AHR showed little to no effect on the efficiency of the chemotherapeutic to kill cells. For both treatment groups, the chemotherapeutic was effectively killing cells, suggesting that the lack of increased chemosensativity was due to some other source other than the chemotherapeutic itself. The most probably conclusion is that doxorubicin acts on the cancer cell independently of a mechanism involving the AHR. Doxorubicin's mechanism is as an intercalating agent of DNA. The intercalating primarily affects the mechanism of topoisomerase II, blocking the transcription and translation of the cancer cell DNA, in turn leading to cell death. Because of its apparent mechanism, it is probable that inhibit the AHR would haven impact on the ability of the intercalating to occur faster or better. Since the AHR is transcription factor, blocking its activity would have no impact in the ability for the topoisomerase II to bind and effectively unwind DNA. It is a probably conclusion that AHR inhibition has little impact of cellular chemosensativity to doxorubicin due to the mechanism by which doxorubicin attacks the cancer cells.

Cisplatin treatment also showed little difference between treatment groups, but most notably showed little cell death in each treatment group over the 0-10.0µM cisplatin dosage range. Because of this lack of cell death is becomes difficult to establish if there is a difference in cell viability between AHR inhibited group vs. the non-inhibited groups. This lack of cell death is most likely attributed to the less pure, metallic cisplatin used for

the purpose of the experiment. The cisplatin administered to patients is a very pure, analytically tested form of the chemo-therapeutic that was substituted with a less pure derivative for the sake of time and cost efficiency. This substitution may have altered the ability of the compound to effectively kill cells as the dosages administered. But given the bit of data that was successfully collected, there is a trend in the data toward statistical significance between the two treatment groups. Similar to doxorubicin, cisplatin targets the cancer call DNA, but via a different mechanism. The cisplatin preferentially bind guanine within the target cells DNA and results in a cross linking of DNA strands. This cross linking inhibits further translation as transcription within the cell and begins the apoptosis process. But, how would AHR inhibition possibly contribute to an increased chemosensativity? Cisplatin generally targets the quickest replicating cells first, therefore there a number of cancer cells left untargeted initially. The AHR inhibition could be contributing to the attack on these cells. Another hypothesis is that the AHR inhibition is preventing transcription of regions of the DNA not inhibited by stand crosslinking. Further research is required to determine if in fact the AHR inhibition is significantly decreasing cell viability and if so, by which mechanism is this occurring.

Following the assays with 5-Flurouracil(5-FU) the AHR inhibited treatment group show a significant decrease in cell viability over the non-inhibited group. This suggests that the AHR inhibition is increasing the chemosensativity of the cells to the 5-FU treatment. For the majority of the doses over the 0.0-100.0µM range, the measure cell viability of the inhibited group was a large percentage lower than the viability of the non-inhibited group. Although the exact mechanism by which this occurs is unknown without further testing, there is a working hypothesis that the 5-FU targets in tandem with the

AHR inhibitor. 5-FU targets and inhibits thymidylate synthase, the enzyme that produces the thymidine, a nucleoside required for replication of DNA. By inhibiting this production, the cancer cells undergo what is considered a thymine less death. Inhibiting the AHR could contribute to this process by inhibiting DNA replication utilizing thymine previously deposited in the cells, decreasing the time required for cell death via the chemotherapy. Further analysis is required to truly understand the contributions of the AHR inhibition to the increased chemosensativity of the cells, but there is a definite conclusion that inhibiting the AHR significantly decreases the cell viability in the presence of 5-flurouracil.

Conclusion

The aryl hydrocarbon receptor plays a number of roles in the development of cancer in individuals but many of these roles are still unclear. Furthering the research conducted on the AHR and the role it pays in chemoresistance and tumor metastasis is vital to predict the best action to treat or prevent cancer. This research has demonstrated that the newly synthesized AHR target compound CB7993113 will actively bind an inhibit the AHR in vivo, effectively decreased the expression of AHR target genes *Cyp1a1* and *Cyp1b1* in both the primary tumor site as well as the liver. This research has also discussed the impacts that AHR inhibition may have on the chemosensativity of cells. Cancer cell treatment with the chemotherapeutic 5-flurouracil is enhanced when the AHR is inhibited. While doxorubicin treatment is not enhanced and it is unclear whether cisplatin treatment is enhanced, the 5-FU data provides insight into the role of AHR in cell

response to chemotherapy. The mystery of the AHR is slowly being uncovered and as

research in this field progresses the battle against cancer continues to stream forward.

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