CHEMOTHERAPEUTIC RESPONSES IN CANINE LYMPHOMA MODELS AFTER TREATMENT WITH THE CHOP PROTOCOL

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ABSTRACT

In both human and veterinary oncology, multi-drug resistance (MDR) is a phenomenon where a cancer gains a cytoprotective effect against chemotherapeutics. Resistance is often witnessed when remitted cancers relapse and become untreatable. As an example, canine lymphomas are notorious for relapsing after treatment with the multi-drug CHOP protocol and response rates for a second remission are substantially lower. While canonical drug efflux transporters have been implicated in the MDR phenotype, other transporters shown to import cytotoxic drugs might also contribute. Recent research has demonstrated that exposure to some chemotherapeutics can result in epigenetic changes to transporter gene expression; this could be a possible route for acquiring a resistance phenotype. What is still unknown, however, is a mechanistic understanding of the chemotherapy-transporter expression relationship. To address this void, we are focusing our research on three questions

1. What are the temporal fluctuations in transporter expression following exposure of canine lymphoma cells to multi-drug regimens?
2. What patterns of epigenetic markers on transporter genes promote altered expression?
3. How does transporter mRNA expression correlate to protein levels in chemo-resistant lymphomas?

We will address each of the questions using genomic and pharmacokinetic methods. This research will provide new mechanistic understandings of chemoresistance, which has useful translational implications.

BACKGROUND

Chemoresistance poses a large clinical challenge to successfully treating cancers like breast, ovarian, and lymphoma. Chemotherapies put tremendous selective pressures on cancers, resulting in many possible modalities to establish resistance (Gottesman, 2002). Importantly, cancers do not need to acquire a permanent resistance phenotype to effectively combat chemotherapies (Yague, 2003). Transporters, including both the ATP-binding cassette (ABC) and solute carrier (SLC) superfamilies, represent one important method cancers can use to establish permanent and transient resistance because of the wide variety of transporters to choose from and their transient expression on cell membranes. Some of the key ABC and SLC transporters have been characterized biochemically, but not much is known about the distribution of these proteins in a membrane, or the regulatory factors that control their expression. It is hypothesized that cancers exploit a combination of these two transporter families to establish and maintain the MDR phenotype.

METHODS

- Immunohistochemistry performed on clinically derived canine lymphomas to determine if SLC transporters are differentially expressed in canine lymphomas. These data suggest that SLC expression (and translation) can be proof-of-concept for the study, and the need for further insight into temporal changes in transporter expression. IC₅₀ value determination now permits us to begin the next step of the project: monitoring of transporter gene expression and epigenetic modifications, but only for three of the four CHOP drugs. Dose response curves for cyclophosphamide, an alkylating agent, have not been generated due to complications with the dosing strategy. Additionally, curves for prednisolone could not be generated due to the lack of cytotoxicity of the drug, which was dosed beyond the clinically relevant equivalent.

RESULTS 1: SLC Transporters are differentially expressed in canine lymphomas

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<thead>
<tr>
<th>Lymphoma Type</th>
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<th>OCT3</th>
<th>OAT3</th>
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Figure 1. Representative images from IHC against listed antibodies in three of the six clinical-lymphoma samples. Table 1. IHC results were tabulated according to clinical sample, highlighting a pattern of differential expression.

RESULTS 2: Dose response can be monitored by fluorescence

Figure 2. Representative images from fluorescent image capture of Oswald (labeled with nuclear-red protein) during 42 hour cytotoxicity assay.

RESULTS 3: IC₅₀ Determination for Oswald and 1771

Table 3. Tabulated data of calculated IC₅₀ values from cytotoxicity assays.

Table 3

<table>
<thead>
<tr>
<th>Drug</th>
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<th>1771</th>
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<tr>
<td>Doxorubicin</td>
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<td>211.3 ng/mL</td>
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<tr>
<td>Vincristine</td>
<td>31.2 ng/mL</td>
<td>&lt;0.73 ng/mL</td>
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<td>Prednisolone</td>
<td>&gt;10 µg/mL</td>
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DISCUSSION

IHC data reveals SLC transporters are differentially expressed in canine clinical lymphoma samples. These data suggest that SLC expression (and translation) can be dynamic amongst a sample population of chemo-exposed lymphomas. This provides a proof-of-concept for the study, and the need for further insight into temporal changes in transporter expression. IC₅₀ value determination now permits us to begin the next step of the project: monitoring of transporter gene expression and epigenetic modifications, but only for three of the four CHOP drugs. Dose response curves for cyclophosphamide, an alkylating agent, have not been generated due to complications with the dosing strategy. Additionally, curves for prednisolone could not be generated due to the lack of cytotoxicity of the drug, which was dosed beyond the clinically relevant equivalent.

Cells will be maintained in either short- or long-term treatment regimens of different combinations of the CHOP protocol. Gene expression will be evaluated by qRT-PCR targeting transporter mRNA and splice variants. These data will begin to reveal temporal changes in transporter expression, and according to hypothesis, will correlate with the extent of treatment that each cell line receives.

REFERENCES


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