Award Number: DAMD17-00-1-0615

TITLE: Role of the Steroid and Xenobiotic Receptor (SXR) in Drug Resistant Breast Cancer

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
The rationale for the work in this proposal was based on the observations that: 1) SXR, a recently-characterized member of the nuclear steroid receptor family, is expressed to varying degrees in normal and neoplastic breast tissues; 2) the anticancer agent Taxol acts as a ligand for SXR and can activate the transcriptional activity of SXR in the context of an SXR-reporter gene construct; 3) Taxol activation of SXR in primary human cells results in increased expression of endogenous human cytochrome P450 3A4 (CYP3A4) and multidrug resistance-1 (MDR1) genes, leading to increased rates of Taxol metabolic inactivation and efflux; and 4) Taxotere, a structurally- and mechanistically-related taxane, is a much less potent activator of SXR in human tissues and taxotere does not induce its own metabolism and extracellular transport. We hypothesized that breast cancer cells that overexpress SXR would be resistant to treatment with Taxol through auto-induction of CYP3A4 and MDR1, while sensitivity to taxotere would be unaffected. We further hypothesized that SXR-mediated induction of CYP3A4 and MDR1 might contribute to clinical multidrug resistance in breast cancer. The objectives of this proposal were to 1) determine if overexpression of SXR in breast cancer cells leads to elevated expression of CYP3A4, MDR1, and other drug resistance-associated genes; and 2) determine if SXR, CYP3A4, MDR1, and other drug resistance-associated genes are coordinately expressed in primary human breast tissues.
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PROGRESS REPORT

Title: Role of the Steroid and Xenobiotic Receptor (SRX) in Drug Resistant Breast Cancer
Number: DAMD17-00-1-0615
Principal Investigator: Susan E. Kane, Ph.D.
Co-Investigator: Timothy W. Synold, Pharm.D.

Introduction:
The Steroid and Xenobiotic Receptor (SRX) is a recently characterized member of the nuclear steroid receptor family that is typified by the Retinoid X Receptor (RXR). SRX is activated by a variety of steroid ligands and one of its most potent activators is rifampicin. The rationale for work in this proposal was based on the observations that: 1) SRX is expressed to varying degrees in normal and neoplastic breast tissues; 2) the anticancer agent Taxol acts as a ligand for SRX and can activate the transcriptional activity of SRX in the context of an SRX-reporter gene construct; 3) Taxol activation of SRX in primary human cells results in increased expression of endogenous human cytochrome P450 3A4 (CYP3A4) and multidrug resistance-1 (MDR1) genes, leading to increased rates of Taxol metabolic inactivation and efflux; and 4) taxotere, a structurally- and mechanistically-related taxane, is a much less potent activator of SRX in human tissues and taxotere does not induce its own metabolism and extracellular transport.

We hypothesized that breast cancer cells that overexpress SRX would be resistant to treatment with Taxol through auto-induction of CYP3A4 and MDR1, while sensitivity to taxotere would be unaffected. We further hypothesized that SRX-mediated induction of CYP3A4 and MDR1 might contribute to clinical multidrug resistance in breast cancer.

Body:
The first aim of our work was to determine if overexpression of SRX in breast cancer cells leads to elevated expression of CYP3A4, MDR1, and other drug resistance-associated genes such as MRP1, MRP2, and SPGP. Towards accomplishment of this aim, we have successfully constructed and transfected SRX expression vectors into MCF7 breast cancer cells and observed SRX expression in 4 independent clones. Work is currently under way to determine if the transfected SRX is functional at inducing downstream effector genes, such as MDR1 and CYP3A4, in response to Taxol and rifampicin. Initial experiments have so far shown no inducible expression in our clones, either at the mRNA level or the protein level. We believe this might be due to tissue-specific differences in expression of essential co-factors or negative regulatory proteins that might be prevent full activity of SRX in some cell types. To address this question, we are currently attempting overexpression of SRX in LS180 colon carcinoma cells, which are known to have basal functional SRX, as well as NIH 3T3 cells and two additional breast cancer cell lines, MDA-MB-231 and MDA-MD-468. We are also screening a panel of 12 cell lines for differential expression of known nuclear receptor coactivators and corepressors. Information about tissue-specific permissiveness for SRX expression and/or function may eventually be useful clinically as potential markers for pharmacologic phenotypes. As part of this aim, we have developed quantitative, real-time PCR methods for measuring SRX, MDR1, and CYP3A4 gene expression. Other genes will also be developed as they are needed. Finally, we have also constructed an antisense SRX expression vector to try to inhibit SRX expression and thus inhibit ligand-inducible drug resistance. Transfection of this antisense construct into SRX-positive cell lines will be initiated shortly.

The second aim of the work was to determine if SRX, CYP3A4, MDR1, and other drug resistance-associated genes are coordinately expressed in primary human breast tissues. We have not made specific progress on this aim. Instead, we have taken a slightly different tack initially. We have developed a method for amplifying tumor-derived DNA from the plasma of cancer patients and we are
using that DNA to look for individual polymorphisms in the SXR gene. Such polymorphisms might eventually be used clinically to identify inter-patient differences in drug metabolism due to differential expression or function of SXR. This work is in its early stages and no outcomes are yet available.

**Key Accomplishments:**

- Construction of 2 SXR cDNA expression vectors that encode a myc-tag epitope at either the amino- or carboxy-terminal end of SXR. Construction of antisense SXR expression vector.
- Transfection of SXR cDNA into MCF7, LS180 and NIH 3T3 cell lines.
- Screening of MCF7 transfected clones and identification of 4 independent clones with SXR expression. Functional characterization is under way.
- Development of quantitative, real-time PCR methods to detect expression of SXR, MDR1, and CYP3A4 mRNA. Analysis of SXR-transfected clones for ligand-inducible expression of CYP3A4 and MDR1 is under way.
- Development of PCR-based method to amplify specific DNA sequences from circulating DNA present in the plasma of cancer patients. Initial look for polymorphisms in the SXR gene.

**Reportable Outcomes:**

**Presentation:**
American Association for Cancer Research, 2001 Annual Meeting

**Manuscripts:**

**Funding received:**
R21 CA91135-01 (NIH/NCI)
“The Steroid and Xenobiotic Receptor (SXR) as a Potential Therapeutic Target in Drug-Resistant Cancer” Principal Investigator: Timothy W. Synold, Pharm.D.
Co-Investigator: Susan E. Kane, Ph.D.
Dates: 04/01/01 to 03/31/03

**Conclusions:**
Work on this one-year grant has allowed us to lay the foundation for future work on the role of SXR in ligand-inducible drug resistance. We have developed several critical tools, including sense and antisense SXR expression vectors; cell lines that express SXR at the mRNA and protein level; PCR-based methods for measuring SXR function; PCR-based methods for isolating tumor-derived SXR DNA from the plasma of cancer patients; collection of probes and cell lines for future analysis of cell line-specific expression of SXR and its coactivators and corepressors. This award also supported the initiation of a collaboration between Drs. Susan Kane and Timothy Synold, which has since led to the successful application for extramural funding from the National Cancer Institute. We expect the outcomes of this totality of work to be the development of basic knowledge about the function and regulation of SXR activity and the ability to make clinical predictions about individual patient responses to chemotherapy.