Erythropoietin and Breast Cancer Progression: An \textit{in vitro} Study \\
Joy Obayemi**, Alyssa Calabro*, Craig Queenan*, David Becker*, and Donna Leonardi** \\
* Bergen County Academies, Nano-Structural Imaging Lab, 200 Hackensack Avenue, Hackensack, NJ 07601 \\
** Bergen County Academies, Biotechnology Lab, 200 Hackensack Avenue, Hackensack, NJ 07601

\section*{Introduction}

Erythropoietin (EPO) is a glycoprotein hormone that is the main regulator of erythropoiesis, the production of red blood cells (RBCs) in mammalian. Advances in biotechnology have made it possible to create recombinant erythropoietins that can induce the same erythropoietic response in animals suffering from anemia as EPO itself. Erythropoietin is produced primarily in the kidneys, but the liver and other tissues may also produce lower levels of it under certain conditions. In normal individuals, EPO is not produced in significant quantities. Under conditions of hypoxia, such as anemia or hypoxia, the production of EPO increases to stimulate the production of red blood cells.

\section*{Materials & Methods}

Cell Culture

MDA-MB-231 and MCF-7 (ATCC) breast cancer cells were cultured in 1:1 DMEM medium supplemented with glutamine (100U/mL), 10% fetal bovine serum, and penicillin/streptomycin, and incubated at 37°C and 5% CO\textsubscript{2}. For experiments at normoxia cells were kept in 21% O\textsubscript{2} environment; hypoxia experiments used 3% O\textsubscript{2}. All cell viability testing was performed using the CellTiter 96® AQueous MTS cell proliferation assay (Promega).

\section*{Erythropoietin Assay}

An ELISA-based EPO assay was performed on cell culture supernatants and plates were incubated at 4°C for 3 hours and then at 37°C for 1 hour. The standard curve was established using a series of EPO dilutions. The assay was run in triplicate and the mean absorbance value was calculated.

\section*{Erythropoietin-Stimulated (EPO) Assay}

Cells were treated with EPO (100U/mL) for 24 hours and then the supernatants were collected and analyzed for EPO using the ELISA-based EPO assay.

\section*{Results & Discussion}

The first phase of this study was to identify the relationship between cancer cell lines, EPO, and chemotherapeutic agents (Cisplatin). In normoxia and hypoxia, it was determined that: exogenous EPO significantly increased cell viability in both normal mammary epithelial cells and breast cancer cells. Cisplatin induced a significant increase in endogenous EPO secretion in hypoxic cells (Figure 1). The effect of this increased EPO production on chemotherapeutic response was then tested. This demonstrated that breast cancer cells treated with a combination of EPO and Cisplatin demonstrated a significantly increased level of cellular death compared to Cisplatin alone (Figure 2). For MDA-MB-231 cells, similar results were found (data not shown). This indicated that EPO treatments significantly increase the efficacy of chemotherapeutic agents.

The next phase of this study examined the anti-angiogenic effect of Lovastatin on these cells. Lovastatin was used as a selective inhibitor of the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), which is an enzyme that plays a critical role in the mevalonate pathway, required for the production of cholesterol. Members of this same statin family of drugs have shown promising results in clinical trials in patients with cancer. It has been suggested that statins may be able to inhibit the growth of tumors by blocking the production of the oestrogen receptor (ER). It has been previously proposed that theLovastatin may block the binding of estrogen to the ER. However, it has also been shown that Lovastatin may induce apoptosis in breast cancer cells.

In this study, breast cancer cell lines were treated withLovastatin, and the suppressor of cytokine signaling (SOCS) pathway was activated. SOCS pathway activation is associated with the downregulation of cytokine and hormone receptors, which can lead to the inhibition of cell growth and proliferation.

Conclusions

Overall, this study shows that EPO does decrease the efficacy of Cisplatin on breast cancer cells. This could be due to the increased production of functional EPO receptors, with the potential to render EPO safe with regard to resistance to chemotherapy. EPO is a potential target for breast cancer therapy. Further studies are needed to determine the mechanism of action of EPO in breast cancer cells and the implications for clinical use.

\section*{References}


\section*{Acknowledgements}

- Dr. Howard Cestero, Superintendent, Bergen County Technical Schools & Special Services
- Edmund Haywood, Technology Director, Bergen County Technical Schools
- Russell Davis, Principal, Bergen County Academies

Presented at Microscopy & Microanalysis 2011
August 7-11, Nashville, TN
Poster Number: 211 Paper Number: 81490

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Concentrations of EPO secreted from breast cancer cells under normoxia and hypoxia. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. normoxia.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure2.png}
\caption{Viability of MCF-7 cells treated with EPO, Cisplatin, and a combination of the two drugs. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. control.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure3.png}
\caption{The effect of Lovastatin treatment on EPO expression in breast cancer cell lines. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. control.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure4.png}
\caption{Lovastatin treatment on breast cancer cell lines under normoxia. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. control.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure5.png}
\caption{Lovastatin treatment on breast cancer cell lines under hypoxia. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. control.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure6.png}
\caption{Lovastatin treatment on breast cancer cell lines under normoxia. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. control.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure7.png}
\caption{TEM micrograph of gold nanoparticle-encapsulated cyclophosphamide in MDA-MB-231 cells. Scale bars = 1 μm.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure8.png}
\caption{TEM micrograph of liposomal carriers containing Lovastatin. B) Viability of breast cancer cell lines after treatment with Lovastatin and theLovastatin-encapsulated liposomal carriers. Bars are means ± STD DEV (n=5). * = p < 0.05 vs. control.}
\end{figure}