

# NORMALIZATION OF BCL-2 FAMILY MEMBERS IN BREAST CANCER BY COENZYME Q10

Cancer is second only to heart disease as the leading cause of death in the United States. Indeed, it is estimated that approximately 178,000 new breast cancer cases were diagnosed in 2007 and 40,000 women will succumb to the disease. The nature of the disease makes it very resistant to chemotherapeutic intervention and radiation. The balance of the Bcl-2 protein family has been implicated as the major contributing factor to conferral of resistance to cancer therapy. Previous work from our research group has demonstrated that Coenzyme Q10 (Q10) is able to significantly decrease Bcl-2 and thereby induce apoptosis in melanoma and prostate cancer. Hence, we postulated that Q10 may have a pro-apoptotic effect in breast cancer. To investigate this hypothesis, we employed the Sk-Br3 and MCF-7 breast cancer lines which exhibit a mutation Her-2/neu and p53 respectively. We examined the effect of Coenzyme Q10 on various members of the Bcl-2 family (bcl-2, bcl-xl, bid, bad, bak, mcl-1, bim, and bax), p53, and caspases 3, 6, 9. All cells were treated for 0–24 hours in the presence and absence of 50  $\mu$ M and 100  $\mu$ M Q10 under physiologic conditions after which total protein was isolated and subjected to Western blot analysis to measure the aforementioned protein products.

The results of our study may provide a template for further investigation into the mechanism of action of mammary oncogenesis while providing support for the use of Coenzyme Q10 as an adjuvant breast cancer therapy. The results showed that there was an upregulation in protein expression of pro-apoptotic members and BH3 subfamily members such as bid, bad, bax, bim, and bak whereas the anti-apoptotic members bcl-xl, mcl-1, and bcl-2 significantly decreased in total protein expression between 4 and 12 hours. Commitment to apoptosis was confirmed by activation of caspase 3, 6 and 9. Conversely, administration of Coenzyme Q10 to mammary fibroblasts did not elicit a significant response on any of the aforementioned intracellular proteins involved in programmed cell death. The data herein suggest that Coenzyme Q10 is able to modulate the various subfamilies of the Bcl-2 family in a manner that restores the apoptotic potential in breast cancer without presenting any adverse effects to normal breast tissue. This provides a template for further investigation into the mechanism of action of mammary oncogenesis while providing support for the use of Coenzyme Q10 as an adjuvant in breast cancer therapy.

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## INTRODUCTION

Women in North America experience the highest rates of breast cancer in the world. It is estimated that 1 in 8 women will be diagnosed with breast cancer, with 1 in 35 eventually succumbing to the disease. A rapidly expanding body of literature deals with other possible adjuvant therapies. Previous research in this lab has shown the potential of Coenzyme Q10 (Q10) in treating cancerous cells. To assess the potential of Q10 on breast cancer, two cell lines were employed by this lab, MCF-7 and SK-BR-3. The MCF-7 and SK-BR-3 cell lines were obtained from the American Type Culture Collection. The MCF-7 line is particularly resistant to many cytostatic drugs. This resistance is due in part to a 47 bp deletion in exon 3 of the caspase-3 gene giving rise to a lack of caspase-3 expression. The SK-BR-3 line exhibits over expression of p185<sup>erbB2</sup> arising from mutations in the erbB2 (also known as her-2/neu) proto-oncogene.

Programmed cell death, or apoptosis, is one of the most important processes in a normally functioning cell. Apoptosis is the cell's own highly regulated and conservative method of dealing with defective cells and maintaining tissue homeostasis. Apoptosis effectively removes damaged, infected, stressed, or starved cells at an estimated rate of 50–70 billion cells a day in the human body. There are two main pathways to apoptosis. The first “intrinsic” pathway is primarily regulated by Bcl-2 protein family members, where intracellular stresses trigger cytochrome c release from the mitochondria which leads to downstream caspase activation. Bcl-2 and its many structurally similar protein family members have been

called the ‘prototypical inhibitors’ of apoptosis. Because of their integral role in intrinsic apoptosis any imbalance can lead to a variety of diseases; under expression can lead to degenerative diseases while over expression can lead to cancer and autoimmune disease. Due to their life or death role in the cell, Bcl-2 family members are currently the targets of many therapies in various disease states. Bcl-2 itself is over expressed in most tumors and all anti-apoptotic Bcl-2 family members are considered to have oncogenic potential. Conversely, the pro-apoptotic members are considered to be tumor suppressors and many mimetics are foci for cancer research.

The focus of this research was to demonstrate the ability of Q10 to mediate over expression of anti-apoptotic Bcl-2 in the SK-BR-3 and MCF-7 cell lines, thus enabling the ability of pro-apoptotic Bcl-2 family members to restore the normal apoptotic capabilities of the cell.

Q10 (2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone) is a lipophilic, fat soluble molecule found in almost every cell in the human body. Q10 plays a pivotal role in the electron transport chain where it is responsible for aiding in 95% of the body's energy conversion via the production of ATP. In addition to its role in energy production, Q10 also possesses antioxidant properties. Q10's capability to confer protection from reactive oxygen species, improve protein/enzyme function and protect DNA has led to a growing body of literature about Q10's therapeutic potential. Q10, also known as ubiquinone, has been shown to have palliative effects on everything from heart disease to neurodegenerative disorders. Some of

the most compelling research demonstrates Q10's effects on cancerous cells and its ability to ameliorate side effects of other therapies. It was the aim of this study to further elucidate Q10's role as a potential breast cancer therapy.

## MATERIALS AND METHODS

ATCC and SkBr3 were grown to 80% confluency in T75 flasks in 5% supplemented DMEM/F12 medium. Both cell lines were treated with a 5% supplemented medium with and without 50 mM and 100 mM Q10 for 4, 8, 12, and 24 hrs. The cells were then collected and lysed for western blot analysis. A 12% Tris-Hcl gel was used to separate the proteins based on molecular weight. The proteins were then transferred to a nitrocellulose paper and incubated with either Bcl-2, Bax, Caspase-3, and Actin antibodies. Using chemiluminescence the expression of protein was analyzed. The protein expression bands were quantified with ImageJ and the values were normalized with the corresponding actin expression. The normal-

ized values were then statistically analyzed using a t-test with  $P < .05$  as significant.

## RESULTS

Both pro- and anti-apoptotic protein levels were measured in the two breast cancer cell lines after Q10 exposure. Protein levels were measured at 4, 8, 12, and 24 hours respectively in order to capture evidence of Q10's normalizing influence on disrupted apoptotic function. In the MCF-7 cell line Bcl-2 levels were seen to significantly drop after only 4 hours of Q10 exposure. Further commitment to apoptosis was seen in the up regulation of Bax and Caspase 3 at this time point. The SK-BR-3 line saw the significant down regulation of Bcl-2 coupled with Bax and Casapase 3 activation at the 12 hour time point.

## DISCUSSION

In the past few years, concern over the rising incidence of breast cancer

reaches ever higher. Education and awareness about this disease are at all time highs and needs for new and novel therapies grow with this tide. Our research demonstrates that Q10 has significant potential as an anti-cancer treatment. Previous research in this lab has shown similar results in melanoma, osteosarcoma and prostate cancer cell lines. In addition to its efficacy, Q10 has shown no cytotoxic effects on normal cells and no known overdose has been documented. Indeed Q10 seems without contraindication or side effect, so important when current chemotherapies are feared almost as much as disease. However, further research into the mechanism of Q10's role in breast cancer needs to be done. RT-PCR may show whether the role is a pre- or post-translational one. Animal models for *in vivo* investigations may provide additional insights on possible outcomes. It is our belief that these future experiments will only strengthen and hasten Q10 from potential treatment to realized therapeutic value.