

INTRODUCTION

The Warburg effect suggests that rapidly dividing tumor cells consume glucose at a much higher rate than normal cells and use glucose to produce lactate rather than undergo oxidative phosphorylation even when oxygen is available (Warburg 1956). Warburg initially suggested that cancer cells have a mutation within the mitochondria not allowing them to perform oxidative phosphorylation. This has been disproved (Moreno-Sanchez 2007). The current theory is that undergoing aerobic glycolysis allows large amounts of glucose to form macromolecular precursors that can be used for cellular proliferation.

We previously showed that both the Ntera-2 cell line and SH-SY5Y cell line downregulate many of the protein subunits of the ETC, perform less oxidative phosphorylation, and yet maintain other aspects of mitochondrial function. These results are consistent with the Warburg effect.

Multiple studies suggest that overactivation of the PI3K/Akt pathway which occurs in most cancers leads to rapid proliferation (Robey 2009). This pathway can be activated many different ways, including estrogen. 17- β estradiol (E2) has been shown to be mitochondria-enhancing and protective (see Figure 2) (Nilsen 2004). The goal of this study was to use E2 to increase oxidative phosphorylation by increasing expression of ETC proteins, which may reverse these aspects of the Warburg effect.

Figure 1

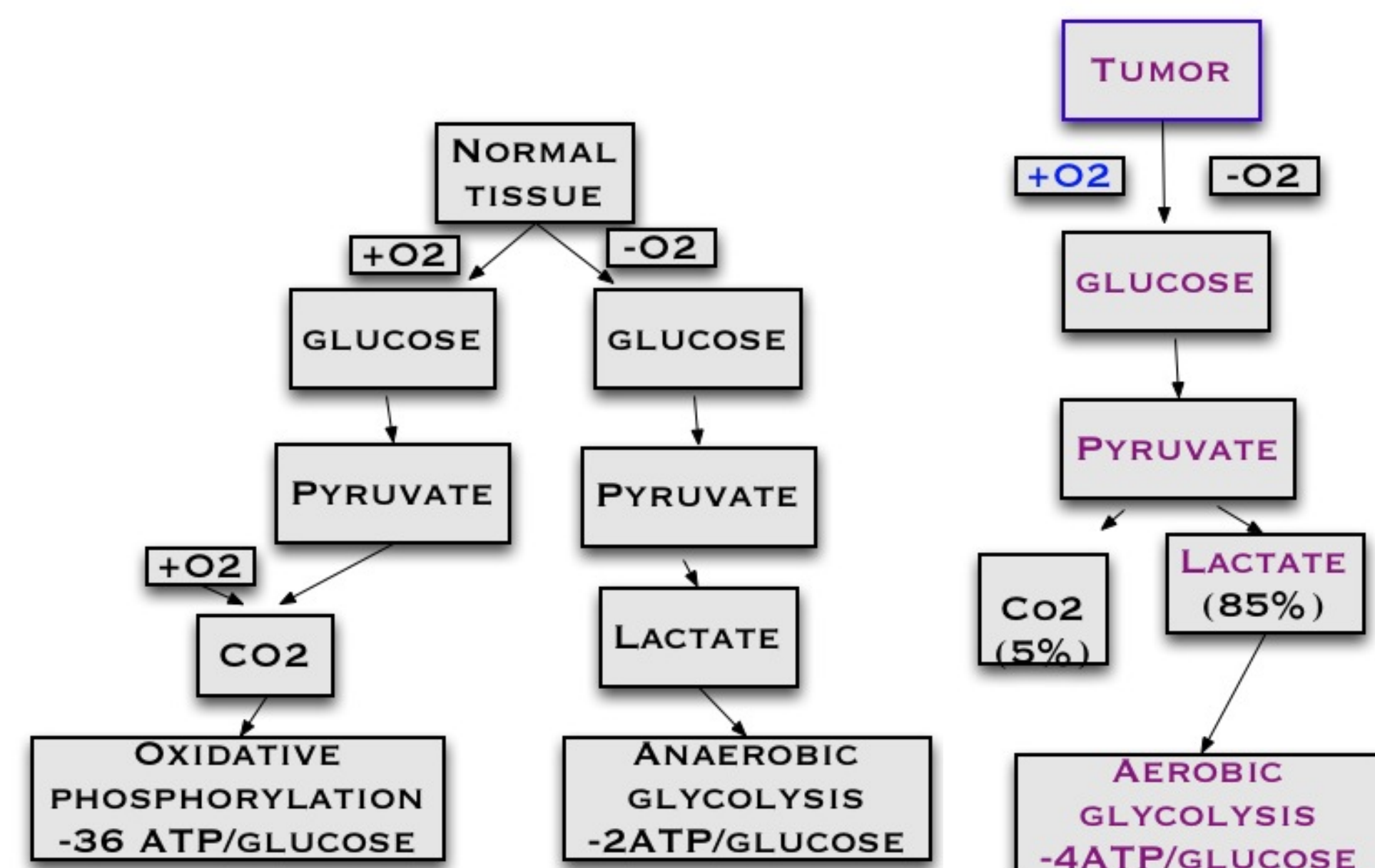


Figure 1: Schematic showing the basic biochemical differences between normal tissue and cancer tissue to explain the Warburg effect.

METHODS

Cell Culture

Human Ntera-2 carcinoma and SH-SY5Y neuroblastoma cell lines (American Type Culture Collection) were used in these experiments. The Ntera-2 line was originally isolated from testicular carcinoma, and the SH-SY5Y subline was derived from neuroblastoma metastasis. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) for nutrients, penicillin-streptomycin to prevent bacterial growth.

E2 treatments

Varying concentrations (0, 10nM, 100nM, 1 μ M) of E2 were added to the samples. Treatments were done for various amounts of time (16-96 hours). 24 hours of E2 treatment has been shown to have a substantial impact on growth of neurons involved in memory (Brinton 1997).

Western Blots

Western blots were performed on both proliferating cell lines as well as RA-differentiated cell lines. In order to determine if we had stimulated any change in the electron transport chain (ETC) subunit protein expression, the blots were probed with OXPHOS antibody cocktail (Mitosciences, Eugene OR) at 1:1000 dilution, and the secondary antibody was anti-mouse Alexa-Fluor 594 at 1:5000 dilution. The subunits of the ETC assessed were ATP synthase, complex I subunit NDUFB8, complex II subunit 30kDa, complex III subunit Core 2, complex IV subunit II. The fluorescent bands were then visualized on UVP blot imager.

RESULTS

We found no indication that the estrogen treatments had the desired effect. There were no apparent differences observed in cells treated with E2 for 16h vs 24h vs 72h vs 96h. There were no differences observed between the cells treated without E2 (the vehicle control) and those treated with the different concentrations (10nm, 100nm, 1 μ M) of E2. Technical difficulties with propagation of the cell lines and troubleshooting the initial blots ultimately consumed the time we had available for further analysis of the hypothesized pathway. It remains possible that E2 or other hormone therapies could induce reversal of aspects of the Warburg effect, under appropriate conditions.

Figure 2

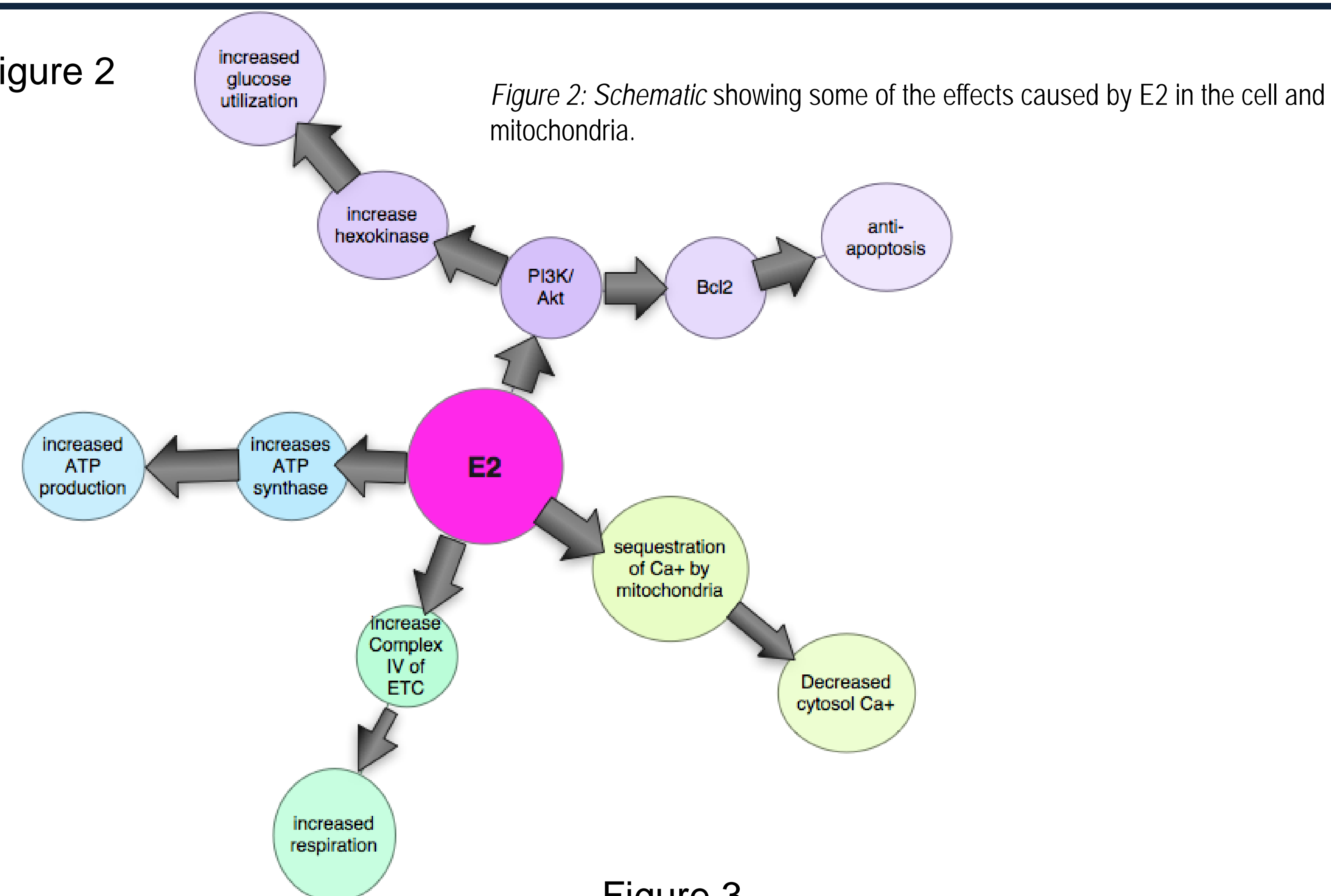


Figure 2: Schematic showing some of the effects caused by E2 in the cell and mitochondria.

Figure 3

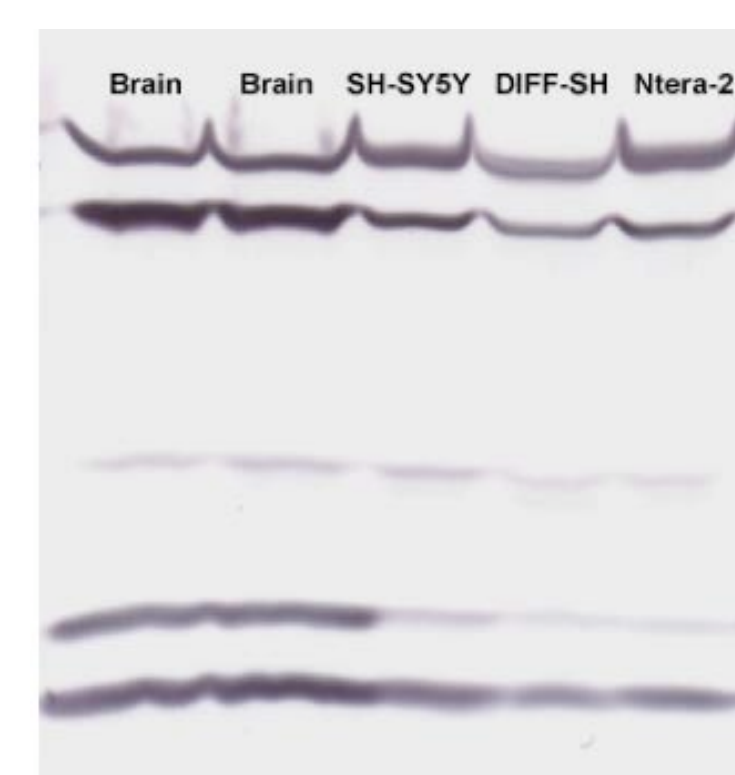


Figure 3: Western blotting and bar graph results showing generalized downregulation of the ETC in cancer lines (proportion of total protein, normalized to % human brain expression) when compared to normal brain.

Figure 3

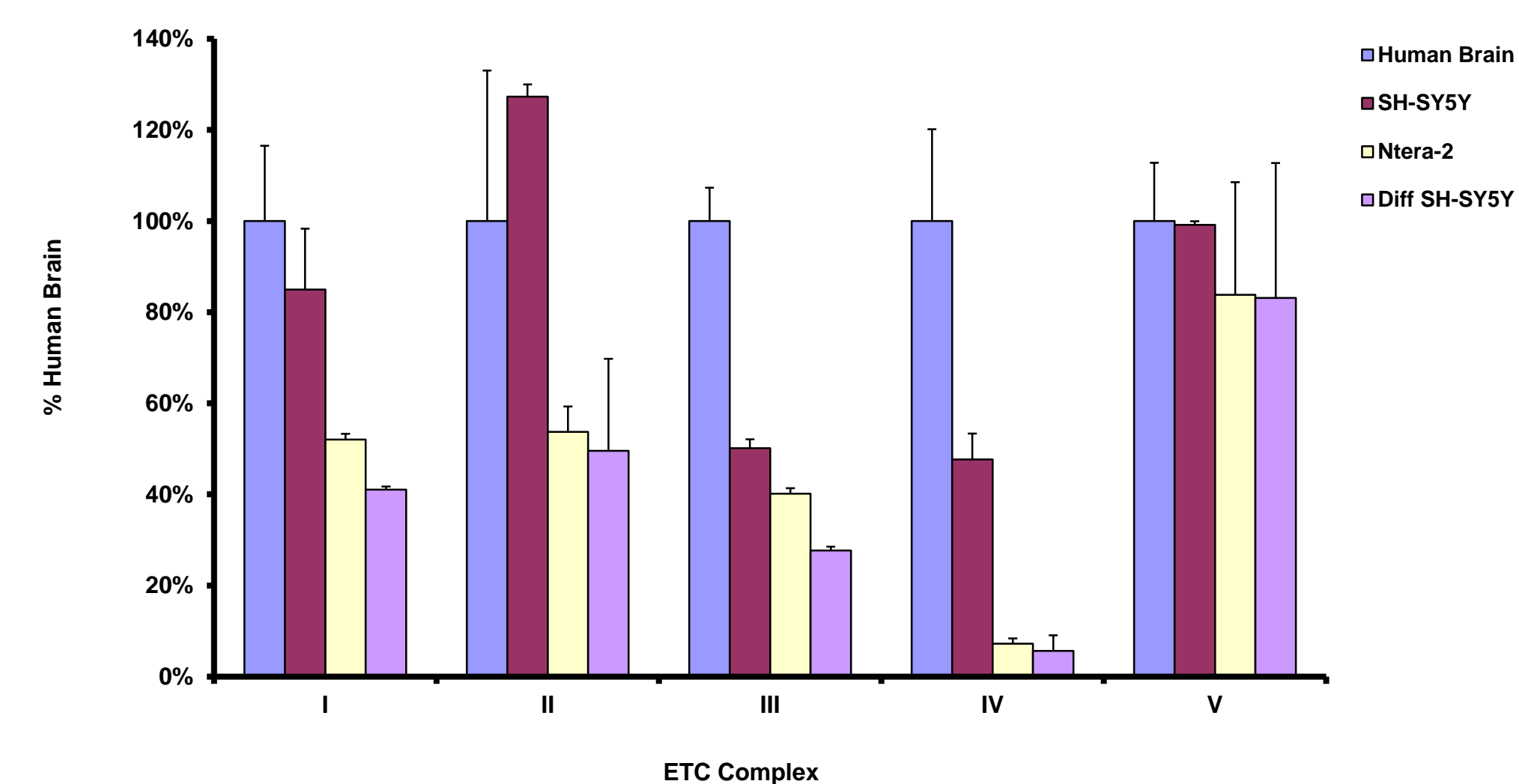


Figure 4

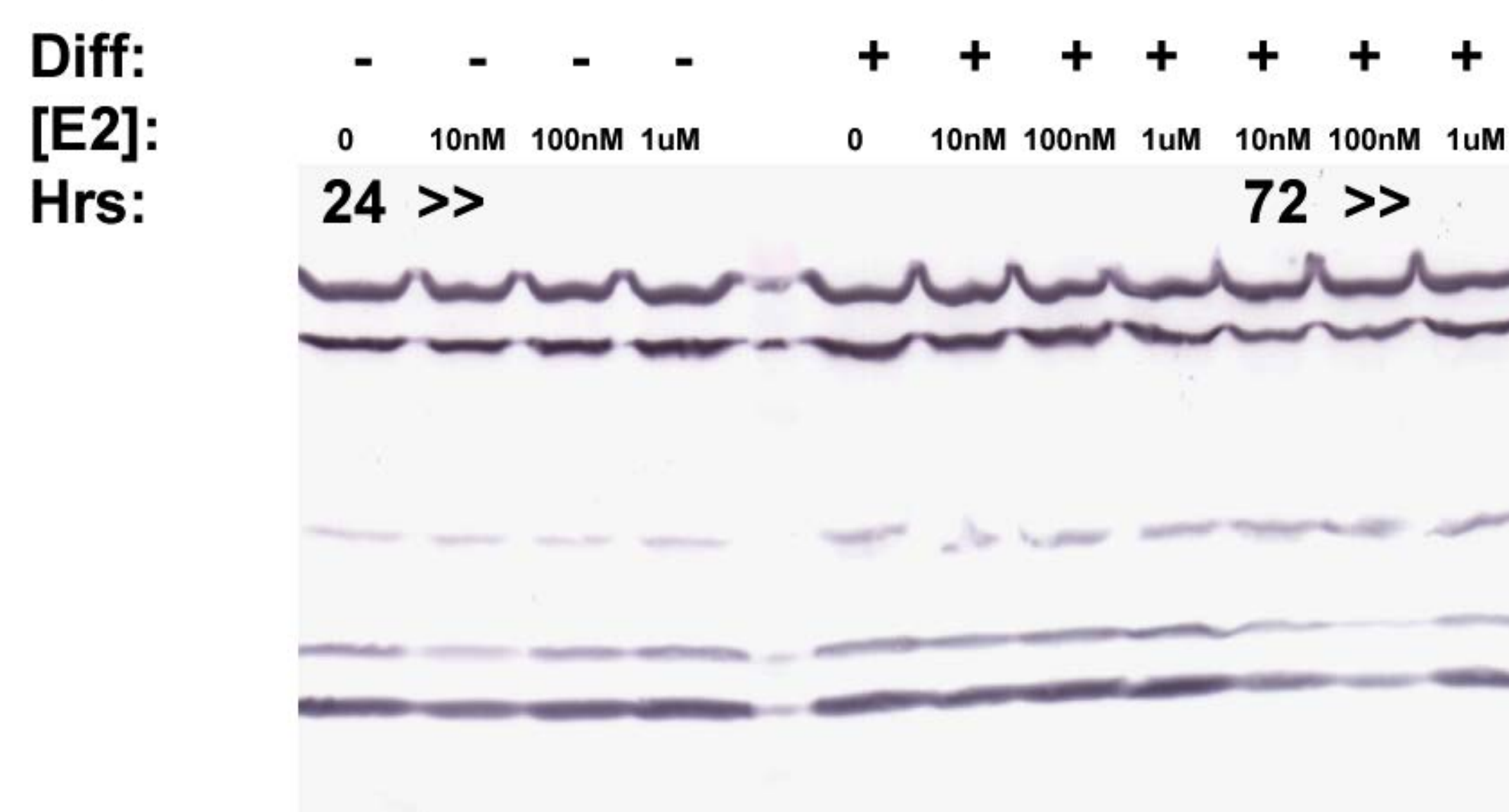


Figure 4: Western blotting results from one of the final experiments testing E2-induced reversal of the Warburg effect. SH-SY5Y cells, proliferating as well as differentiated, were harvested after 24 or 72 hours of E2 treatment at various concentrations. Neither increased E2 concentration, from 10 nM to 1 μ M, nor increased time of treatment significantly increased the expression of the ETC subunits assessed in proliferating or RA-differentiated cells. Bands are as follows: Uppermost: ATP synthase subunit alpha, approx 55kD; Complex III subunit Core 2, approx 48kD; Complex II subunit 30kDa, approx 30kD; Complex IV subunit II, approx 24kD; Complex I subunit NDUFB8, approx 20kD.

CONCLUSION

Our results demonstrated a failure of 17- β -estradiol (E2) to enhance the expression of ETC protein subunits in our cancer cell lines. A number of technical difficulties may have confounded our interpretation, and further investigation would be required to determine whether our hypothesis, that activation of the Akt/PI3K pathway can reverse these aspects of the Warburg effect, is correct or false. Considerable work remains in order to determine whether this bioenergetic effect can be reversed, in vitro and ultimately in vivo, and whether doing so would have a beneficial effect on patient outcomes.

REFERENCES

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