

Abstract

Purpose: The objective of this experiment is to look at the changes in the ErbB receptor tyrosine kinase family within cells exposed to Arsenic. We are looking for differences in expression (or amount) of protein and variation in activity levels.

Methods: A non-malignant human bronchial epithelial cell line, Beas-2B cells were exposed to 100 nM sodium arsenite for 24 weeks. Multiple cultures of cells were maintained separately for 24 weeks. The negative control used is human Telomerase Corneal Epithelial Cells (hTCEpi cells) with no ligand treatment. The positive control used is Beas-2B cells grown in arsenic but treated with ligand. Immunoblots were performed to measure the expression and activity of EGFR, ErbB2, ErbB3, and pY1068. Three Alamar Blue assays were performed to test Beas-2B cell sensitivity to Epidermal Growth Factor (EGF) ligand and Transforming Growth Factor- α (TGF- α) ligand in amounts of 1, 10, and 100 ng/mL. This process was also done with a sample exposed to 12 weeks of As.

Results: Western blots show chronic arsenite exposure induces overexpression of EGFR under both conditions of EGF treatment. The amount of ErbB2 decreases when the cells are treated with arsenic, and ErbB3 levels are unchanged. ErbB4 was not tested for due to unreliable antibody control. The 12-week treated samples show an increase in ErbB2 and a decrease in ErbB3. pY1068 increase only when looking at 12-week arsenic treated to 24-week arsenic treatment. The Alamar Blue assays show a higher sensitivity of the 24-week passage-matched cells to ligand treatment.

Conclusions: Chronic arsenite exposure on Beas-2B cells only significantly impacts EGFR overexpression repeatedly. There was slight decrease in ErbB2 amount from the passage-matched to the arsenic treated cells in multiple times as well. pY1068 phosphorylation was detected to be about equal multiple times but in some immunoblots more activity was present in Beas-2B cells treated with arsenic.

Introduction

Chronic exposure of arsenic (As) is associated with the development of skin and lung cancer. However, the exact mechanism by which arsenic induces carcinogenesis is unknown. Previous studies (Kim, C.) have shown that acute exposure of arsenic induces overexpression of Epidermal Growth Factor Receptor (EGFR). The ErbB or EGF family is made up of 4 receptor tyrosine kinases: {ErbB1; HER1; EGFR}, {ErbB2; HER2}, {ErbB3; HER3}, {ErbB4; HER4}. Under balanced conditions, these proteins play key roles in relaying signals that regulate cell proliferation, mobility, differentiation, and apoptosis. These proteins are associated with tumorigenesis and cancer propagation. With arsenic being ranked number 1 in the Agency for Toxic Substances and Disease Registry Substance Priority List, studying this pathway could lead to big discoveries in not only lung, but also skin and bladder carcinogenesis as well. ErbB1 or EGFR gene amplification, mutation, and rearrangement characterizes many types of cancer. Previous studies have shown that arsenic exposure increases EGFR expression and activation, but the mechanism still remains elusive. It is activated by specific ligands, such as EGF and TGF- α . Over-activation and hyper-activation of ErbB family members causes increased downstream signaling and activates proteins associated with cell proliferation, survival, migration, and tumorigenesis.

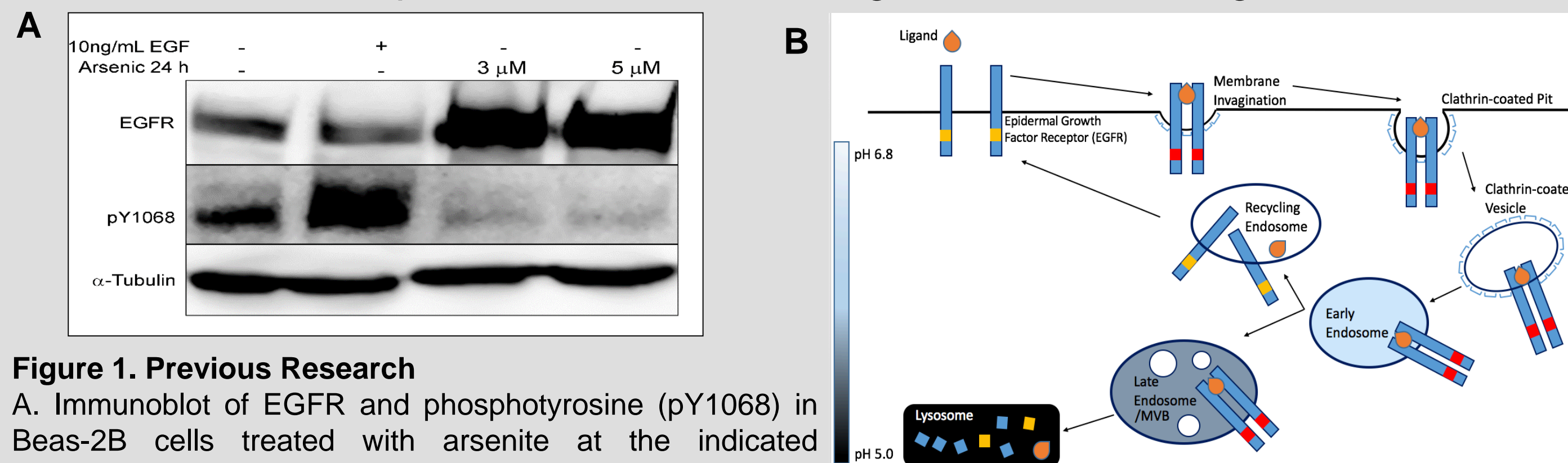
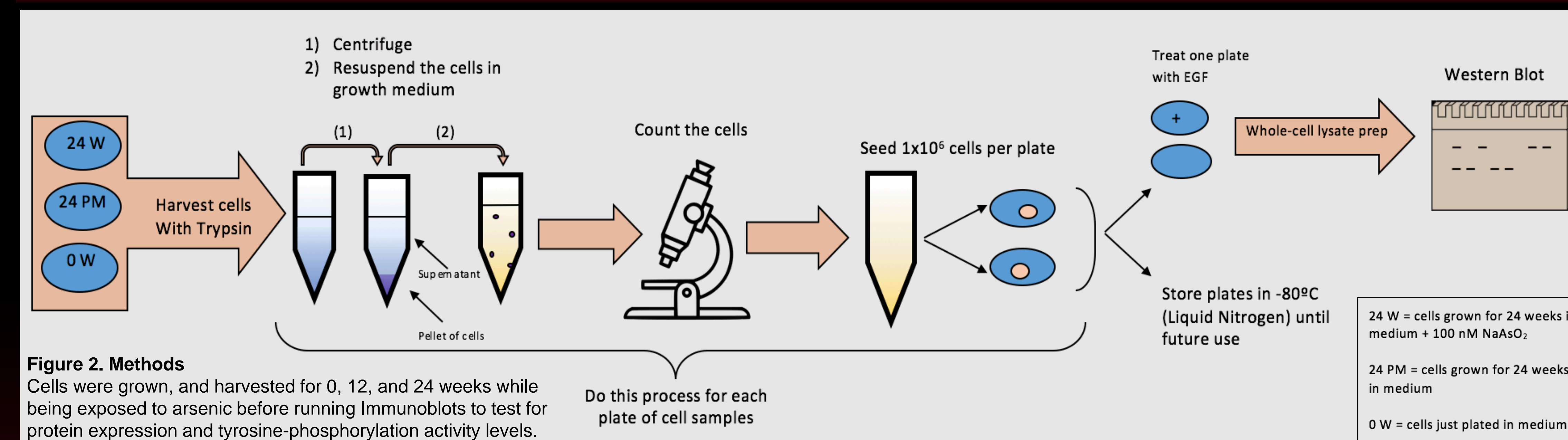


Figure 1. Previous Research
 A. Immunoblot of EGFR and phosphotyrosine (pY1068) in Beas-2B cells treated with arsenite at the indicated concentrations for 24 hours shows increased EGFR levels in cells treated with arsenite. (Kim, C.) B. EGF receptor trafficking pathway. Receptors can either be recycled back to the membrane or targeted for degradation in the lysosome by ubiquitination.

Hypothesis

Our overarching hypothesis is that chronic exposure of low levels of arsenite disrupts the trafficking pathway of the ErbB family receptors.

Methods



Results

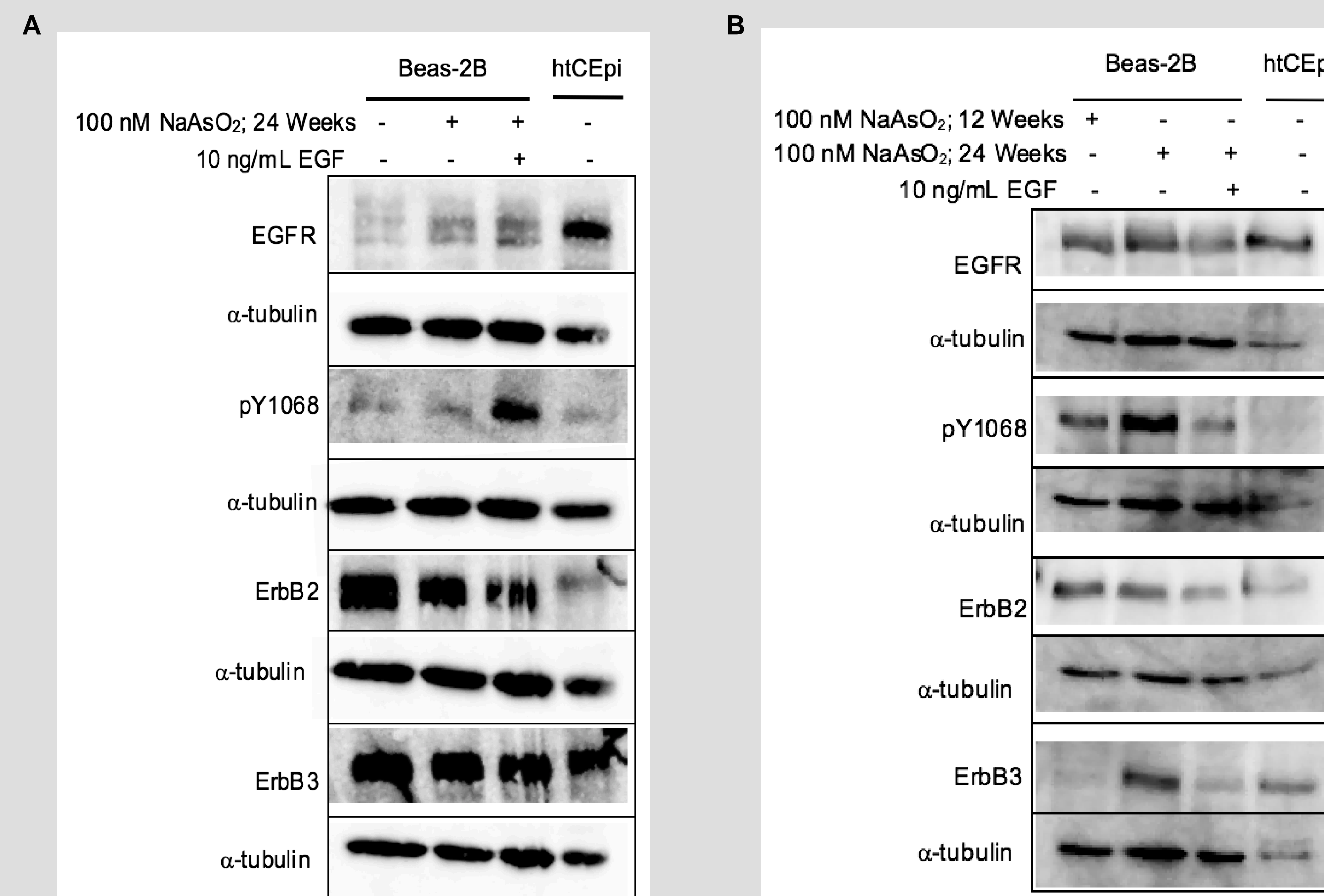
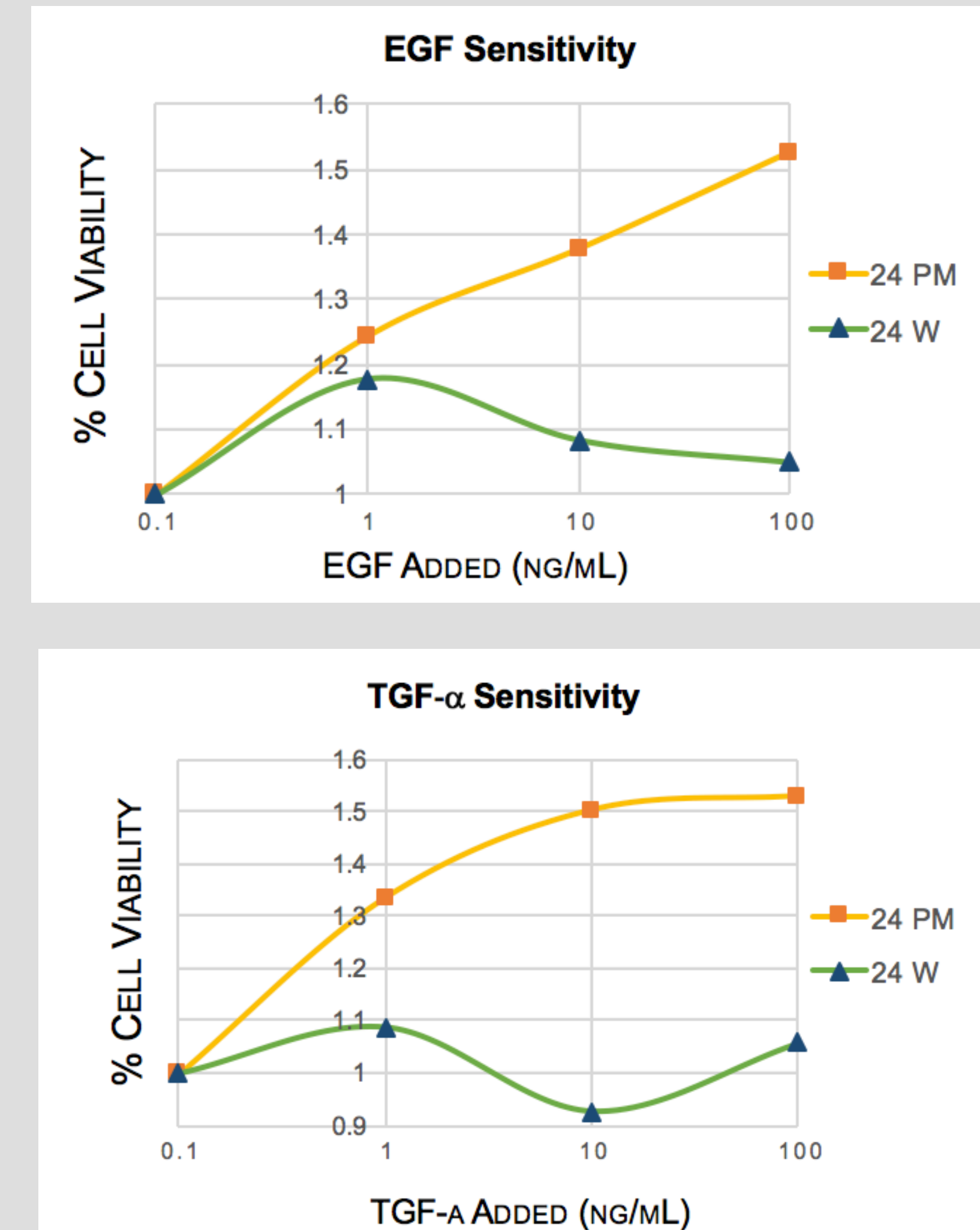


Figure 3. Immunoblots of 24- and 12-week treated cells with arsenic.
 A. The immunoblot shows data from harvested cells of 24 weeks untreated, 24 weeks treated with arsenic, and a positive and negative control (htCEpi cells). B. The immunoblot shows data from harvested cells of 12 weeks arsenic-treated, 24 weeks treated, and a positive and negative control. Both blots were run with the same 24-week arsenic treated sample as well as the two control samples.

Results cont.



Discussion and Future Directions

Many studies focus on the effect of acute exposure with high levels of arsenic. Our study focuses on chronic arsenic exposure because it is more environmentally relevant to the conditions observed in human blood-levels of individuals who live in arsenic-contaminated regions or who heavily rely on arsenic-contaminated water supplies. Present studies highlight the biological effect of chronic exposure to low levels of arsenic and present possible mechanisms of arsenic-mediated overexpression of EGFR, including alteration in the EGFR endocytic distribution, changes in the levels of each type of ErbB receptor, and variation in sensitivity to different endogenous ligands. Results show that in Beas-2B cells exposed to arsenic for 24 weeks, EGFR amounts increase while ErbB2 and ErbB3 both decrease. In the same cells exposed to arsenic for 12 weeks, there is a decrease in ErbB3, although other harvested samples gave different variability. There is a slight increase in ErbB2 and a decrease in phosphorylation levels.

The Alamar Blue assays follow a dose-response like curve for the passage-matched cells. For the 24-week arsenite treated cells, both curves peak at a 1 ng/mL ligand treatment and then decrease after that. This could be due to receptor down-regulation in the 24-week treated cells.

The proteins we study help to locate new biomarkers that can be used in the diagnosis, prognosis, and treatment of cancer. Learning the underlying mechanisms now can open future doors for better understanding of not lung carcinogenesis alone, but also formation and progression of other cancers throughout the body.

Acknowledgements

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