**Regulation of ORMDL3 Gene Expression through the ESK/CREB Pathway**

**I. Introduction**



**Figure 1 Asthma in the U.S. by Age and Gender from 2001-2009.** A gradual increase in the percentage of those afflicted with asthma in the population can be observed, with a large amount of the affected population being made up of children. Adapted from the NIAID (reference 5).

 Asthma is a chronic inflammatory disease that is associated with airway obstruction, inflammation, and hypperreactivitiy. Recent surveys have shown that about 5-16% of the world’s population is affected by asthma; the United States remains to be one of the countries with the highest asthma rates, and the rates continue to increase each year with a significant amount of the affected population being children (Figure 1) (Martinez and Vercelli 2013). Inhaled corticosteroids is the common treatment when presenting with wheezing and other asthmatic symptoms, but community studies have shown that compliance to daily therapy is only about 20%.

 Vitamin A supplementation has been a recent treatment under consideration for asthma. It has been known to play a role in immune response and in maintaining the epithelium of the airway. Though there have been consistent findings of decreased levels of vitamin A in asthmatic patients, treatment through vitamin A supplementation remains controversial; supplementation at an early age is not associated with the decreased risk for the onset of asthma, and in other cases, supplementation at an early age cause the release of cytokines that produce the asthmatic response. Over 150 genes have been associated with producing these responses, one of which is ORMDL3, where ORMDL3 is more explicitly involved with the onset of childhood asthma (Zhuang et al. 2013).

Orosomucoid 1-like 3 (ORMDL3) is a gene that is a part of the ORMDL family that is found in humans. While ORMDL1 and ORMDL2 are both known to be expressed throughout adult and fetal tissue, ORMDL3 is specifically expressed in bronchial epithelial cells. The gene encodes a trans-membrane protein that binds to a pump regulating the calcium ion (Ca2+) within the sarco-endoplasmic reticulum (Ha et al. 2013).. Altering the pump’s activity, in turn, affects the unfolded protein response, which plays a key role in the development of inflammatory diseases like asthma (Kang et al. 2012). Earlier this year, a study found that all-trans retinoic acid (ATRA), a metabolite of vitamin A, was capable of increasing the expression of ORMDL3.

 A number of additional signals were involved in allowing ATRA to eventually express ORMDL3. The expression of ORMDL3 is triggered when a cAMP response element (CRE) found in the promoter region of the gene binds to a cAMP response element-binding protein (CREB) (Zhuang et al. 2013). While there are a number of kinases known to bind to CREB, Zhuang et al. (2013) found that protein kinase A (PKA) phosphorylated CREB and then allowed for ORMDL3 expression. When inducing cells with ATRA, ORMDL3 expression also increased accordingly, and they concluded that PKA must be the connecting factor between ATRA induction and ORMDL3 expression. Of the other kinases capable of activating CREB, extracellular signal-regulated kinase-1 and 2 (ERK1/2) is known to activate CREB via phosphorylation (Aggerwal et al. 2013). However, it is not known if ATRA-activated ERK1/2 will also increase ORMDL3 expression. The purpose of this experiment will be to determine whether or not ERK1/2 also induces ORMDL3 to further understanding of how vitamin A supplementation may be used as a treatment to prevent the onset of childhood asthma.

**II. Experiment**

 Three cell types will be used in this experiment: mouse embryonic fibroblast cells (NIH3T3), human bronchial epithelial cells (BEAS-2B), and human type-II alveolar lung epithelium cells (A549); each of the cell types are representative of the different environments in which ORMDL3 is found to be expressed. The cells were plated onto 96-well plates, where about 15,000 cells were in each well. Each of the cell types were divided into four groups: PKA, anti-PKA, ERK1/2, and anti-ERK1/2; the first two groups were to serve as a control, and anti-PKA was to represent the inhibition of PKA by treating the cells with 15 µM of H-89. U0126 was used to inhibit ERK1/2 in the anti-ERK1/2 group.The cells were then treated with 1 µM of ATRA and left to incubate for 18 hours (Figure 2). Afterwards, the cell proteins were extracted using the Total Protein Extraction Kit, and the western blot was initiated.



**Figure 2 ORMDL3 Production in Presence of PKA**. Measurement of ORMDL3 expression shows that 18 hours is the optimal amount of time needed to produce significant protein. Adapted from Zhuang et al. (2013) (reference 7).

 A western blot is a test commonly done to detect for the presence of a specific protein. The first part of the test involved the separation of various proteins by mass by using gel electrophoresis. A 12% SDS-PAGE gel was used to run the test. Afterwards, the proteins were transferred over to the nitrocellulose membrane through the electrophoretic transfer method. The membrane was then blocked using TBS to prevent binding between the membrane and the antibodies to be later added. Following incubation with the blocking agent, the membrane was blotted with 2 µM of ORMDL3 antibody, E-15; the antibody was obtained through Santa Cruz Biotechnologies and chosen over N-14 for its effectiveness based on data analysis. The membrane was allowed to incubate in the antibody solution for 30 minutes with little agitation. The blot was then stripped and re-probed using β-tubulin as the loading control to quantify the ORMDL3 protein present. Analysis of the blot was done using the Odyssey System with infrared absorption to also allow for measurement of the luminescence, from which the concentrations of the protein could be derived.

**III. Discussion**

 Should the experiment be completed without issues, the western blot should show that ORMDL3 protein is present in the PKA and anti-ERK1/2 lane, and not in the remaining two lanes; the bands for the loading control, β-tubulin, should be consistent across all four lanes. Measurement of the ORMDL3 protein should show that there is a higher concentration in the PKA lanes as opposed to the anti-ERK1/2 lanes. If a treatment were to be developed solely using knowledge of these two kinases, this experiment serves to show that a vitamin A supplementation would be best given alongside an ERK1/2 inhibitor. However, because the previous study done observes ERK1/2 activating CREB with two other kinases, it is not known if only inhibiting ERK1/2 will be effective. Further studies should be done to determine if the three kinases produce a synergistic response similar to PKA. Additionally, other CREB activators, and combinations of them, should also be tested to obtain a clearer understanding of the pathway to then develop an effective treatment to stop the onset of childhood asthma using vitamin A supplementation.

 The bulk of the potential problems will arise from the reliability of the data obtained from the western blot. Because this test is extremely sensitive, it is likely that too much or too little of the antibodies could be detected, thus leading to falsehood in the data. This could be corrected by altering the incubation time after finding the optimal time or simply be doing another trial of the experiment. Transferring the gel onto a nitrocellulose membrane and then later stripping the membrane may prove to be a problem as nitrocellulose is more fragile. Should this be the case, the alternate membrane, polyvinylidene fluoride (PVDF), should be used as it is capable of handling stripping.

 The main limitation of this study is the lack of kinase diversity. If this experiment were to become more expansive, the kinases coupled with ESK1/2 in Aggerwal et al.’s study, RSK and PKC, would have also been incorporated. Additionally, this mechanism is unusual in that it is appears to be independent of the retinoic acid receptor. Further testing on this key point would help define the mechanism and perhaps narrow down the kinases that are involved in the pathway. Finally, the experiment does not measure the ORMDL3 mRNA produced to determine whether or not an intermediate is being produced at all in cells that eventually did not produce any protein. Exploration of all of the aforementioned may help designing a vitamin A supplementation treatment that produces less contradicting results.

**III. References**

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