Cytokeratin-8/18 Intermediate Filaments and their Role in Regression of the Bovine Corpus Luteum

Nicolle E. Young, Sarah E. Grieve, David H. Townson
Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH

Abstract
Cytokeratin-8/18 (CK8/18) is a filamentous protein within cells known to influence expression of cell surface receptors and susceptibility to hormone-induced cell death (apoptosis). Specifically, CK8/18 filaments sequester cell death receptors within the Grb2 apparatus, thus preventing expression on the cell surface and blocking apoptosis. In our current study, the goal was to initially determine whether CK8/18 filaments can be pharmacologically disrupted without affecting cell viability. To do this, acrylamide was used in time- and dose-response experiments to transiently disrupt CK8/18 filaments in human cervical cancer cell line (HeLa cells). Fluorescent detection of CK8/18 filaments revealed that HeLa cells incubated in 10 mM acrylamide for 12 hours had disrupted cytoskeletal structure. However, nuclear staining of the cells indicated that both control and acrylamide-treated cultures remain viable. Measurement of mitochondrial dehydrogenase activity further confirmed that there was no overt negative effect of acrylamide treatment on the cells of the bovine ovary, our plan is to next disrupt CK8/18 filaments in luteal cells of the bovine corpus luteum and assess their susceptibility to hormone-induced apoptosis. Regression of the corpus luteum is a major factor influencing reproductive health in dairy cows, and as such is the core of the dairy industry. Ultimately, our goal is to achieve a better understanding of the physiological mechanisms that regulate apoptosis of ovarian cells.

Introduction
In the dairy industry, reproductive health and management of dairy cattle is an economic concern. Fertility is controlled in part by an ovarian structure known as the corpus luteum (CL), which is required for the maintenance of pregnancy1. Luteal regression or death of cells (apoptosis) of the CL results in embryonic loss and infertility2. Recently, we have determined that cells of the CL (luteal cells) contain filamentous proteins comprised of cytokeratin 8/18 (CK8/18). In other tissue systems, CK8/18 filaments are postulated to protect cells from hormone-induced apoptosis3, 4. Perhaps CK8/18 filaments within luteal cells serve a similar function to protect the CL from luteal regression.

Here, we have developed a cell model system using a human cancer cell line (HeLa cells) to initially test the hypothesis that CK8/18 filaments protect cells from hormone-induced apoptosis. The objective of the current study was to determine if CK8/18 filaments can be pharmacologically disrupted in HeLa cells without affecting viability. Eventually, we hope to translate these methods to luteal cells of the bovine CL and determine whether CK8/18 filaments serve a protective role against luteal regression.

Materials and Methods

Cell Culture
Aliquots of HeLa cells, a human cervical cancer cell line, were used in these experiments. The HeLa cells were grown in 8-well chamber slides for the fluorescent detection of CK8/18 filaments and nuclear staining, and in 96-well plates for measurement of mitochondrial dehydrogenase activity. In all experiments, cells were cultured in Eagle's Minimum Essential Medium (MEM) with gentamicin and 10% fetal bovine serum. The cells were incubated in a 37ºC humidified incubator of 95% air and 5% CO2.

Fluorescent Microscopy
Acrylamide, a protein disrupting chemical, was used in time- and dose-response experiments on 70% confluent HeLa cells. The experiments involved 5 mM, 7.5 mM, and 10 mM acrylamide dosages for 6, 12, and 24 hours. The HeLa cells were fixed in 1% paraformaldehyde in PBS and stored in 70% ethanol at 20ºC prior to staining. FITC-labeled CK18 antibody was applied and incubated in the dark at room temperature for 1 hour, followed by a DAPI counterstain. Fluorescent microscopy was used to assess the cytoskeletal structure, specifically, the cytokeratin filaments with FITC-labeled CK18. DAPI was used to counterstain the nuclei to determine structural integrity.

Viability Assay
Following 12 and 24 hour 7.5 mM and 10 mM acrylamide treatments, a viability assay was performed based upon mitochondrial dehydrogenase activity. After 12 hours the acrylamide was removed and the viability assay performed. At this point, half the cells had fresh MEM added and the plate was allowed to incubate for another 12 hours. This was performed to further assess cell viability and determine if cytoskeletal structure could be repaired after acrylamide disruption. A plate reader was used to measure and quantify dehydrogenase activity after both 12 hour periods. A comparison was made between controls and treated cells to determine effects of the acrylamide and to ascertain cell viability.

Summary
- HeLa cells treated with 7.5 and 10mM acrylamide exhibited disrupted CK8/18 cytoskeletal structure, and little cell death, as evident by fluorescent microscopy (FITC and DAPI staining).
- Treatment of 10mM acrylamide had no effect.
- Measurement of mitochondrial dehydrogenase activity indicated an average of 32% cell death following 7.5 and 10 mM acrylamide treatment (12 hours).

Conclusion
These preliminary results suggest that acrylamide is an effective disrupting agent of CK8/18 filaments and cytoskeletal structure. However, further investigation is needed to resolve issues concerning effects on cell viability prior to testing the hypothesis that CK8/18 filaments protect cells from hormone-induced apoptosis.

References

Acknowledgements
Jessica Cherry and David Townson
Department of Animal and Nutritional Sciences, University of New Hampshire