The endocytic pathway plays a major role in the regulation of tight junction remodeling during nutrient starvation in jejunal IPEC-J2 cells

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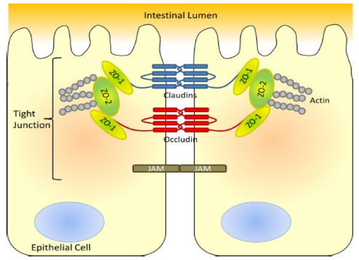
Introduction

Intestinal epithelial cells are involved in nutrient absorption and also form a barrier to prevent passage of antigens into blood circulation. Adjacent epithelial cells are tightly bound by tight junction proteins (TJP) which consist of transmembrane proteins (claudin family protein and occludin), as well as cytoplasmic components, such as zonula occludens (ZO)-1, ZO-2 and ZO-3. Approximately 50% of pigs do not consume any feed during the first 24 h post-weaning. There is growing evidence that low post-weaning feed intake in piglets is associated with disruption of TJP, compromised epithelial cell function and reduced growth performance. However, the underlying mechanism by which the TJP are dysregulated in response to nutrient deprivation remains poorly understood.



Objective

The objective of this study was to use IPEC-J2 cells as an in vitro model to elucidate the underlying mechanism involved in the tight junction remodeling during nutrient deprivation.

Materials and Methods

Experimental design:

IPEC-J2 cells were nutrient starved in Krebs-Ringer bicarbonate (KRB) buffer for 0, 1, 2, 3, 6, 9, 12 and 24 h.

Pharmacological inhibition:

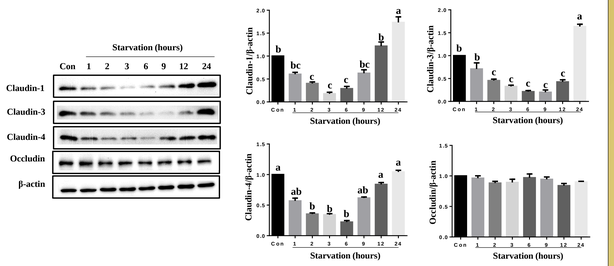
Bafilomycin (BAF), (10 nM), MG132 (50 μM) and cycloheximide (CHX) (200 μM) were used to inhibit lysosome, proteasome and protein synthesis, respectively, by addition to the KRB media for indicated starvation time. IPEC-J2 cells were pretreated with Wortmannin (100 nM), Dynasore (100 mM), Pitstop 2 (10 μM) and Genistein (200 μM) in the cell culture media for 1 hour prior to starvation to inhibit autophagy, dynamin, clathrin or caveolae-dependent endocytosis, respectively.

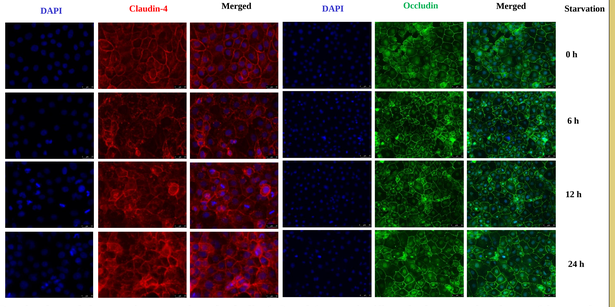
Data analysis

PROC GLM procedure of SAS was used to analyze data. Means were separated using Tukey's multiple comparison. P-value for test of significance was set at 0.05.

Results

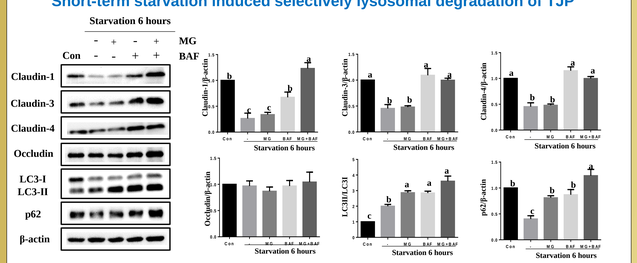
Nutrient starvation induced tight junction remodeling in IPEC-J2 cells.

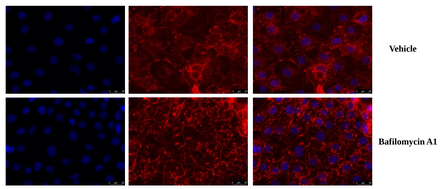




Results showed the protein expression of caludin-1, 3 and 4 were significantly downregulated up to 6 hours of starvation and then increased thereafter, whereas occludin was not affected by starvation challenge

Short-term starvation induced selectively lysosomal degradation of TJP

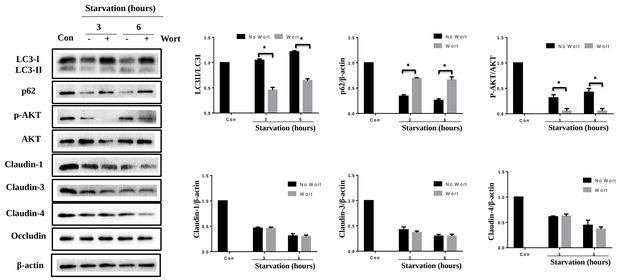


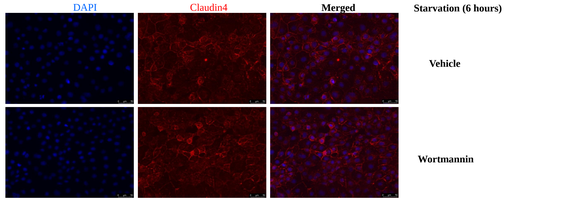


Upon 6 hour of starvation, the protein level of claudin-1, 3 and 4 were significantly reduced and this reduction was reversed by BAF but not MG132 in the starvation media.

Results continued….

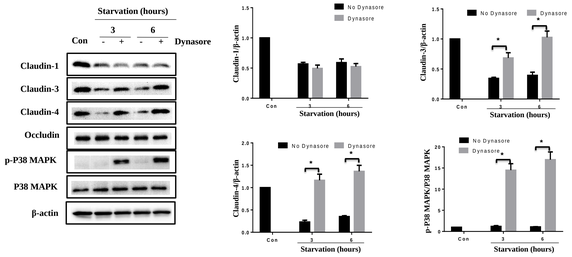
Lysosomal degradation of TJP was not mediated by autophagy-lysosomal pathway

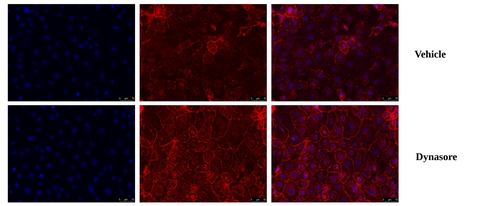




Results showed that the ratio of LC3-II to LC3-I was decreased and p62 was significantly accumulated in wortmannin treated group compared to cells in starvation alone, which indicated the autophagy was inhibited when cells were treated with wortmannin. However, the degradation of these TJP was not blocked by autophagy inhibition

Lysosomal degradation of TJP was mediated by endocytic pathway

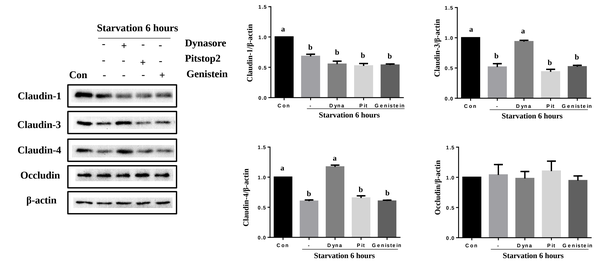




Results showed the degradation of claudin-3 and 4 up to 6 hours of starvation was reversed by dynasore supplementation, whereas the degradation of claudin-1 during short term starvation was not affected.

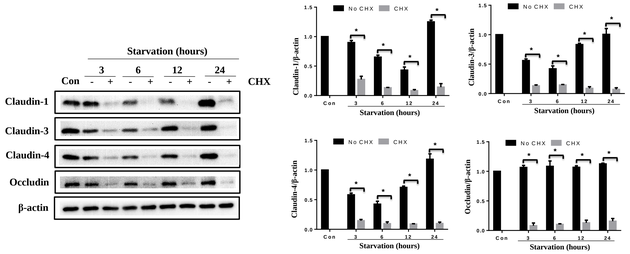
Results continued….

Lysosomal degradation of TJP was not mediated by clathrin or caveolae-dependent endocytosis



The role of two major dynamin dependent endocytic pathways in degradation of claudin-3 and 4 in starvation-treated IPEC-J2 cells was analyzed using clathrin dependent endocytosis inhibitor (pitstop 2) and caveolae dependent endocytosis inhibitor (genistein). Results showed that the degradation of claudin-3 and 4 was not mediated by clathrin or caveolae-dependent endocytosis, indicating that the endocytosis of claudin-3 and 4 was via a dynamin dependent but clathrin and caveolae independent pathway.

Prolonged starvation induced re-synthesis of TJP



The endocytosed protein could be either degraded in the lysosome or recycled back to cell surface. To clarify the mechanism involved in the upregulation of claudin-1, 3 and 4 during long-term starvation period (12 and 24 hours), protein translation inhibitor (CHX) was supplemented in the starvation media. Results showed that the upregulation of TJP were significantly abrogated upon the CHX supplementation at each indicated starvation time, indicating that long-term starvation induced de novo synthesis of claudin-1, 3 and 4 rather than the increased rate of recycling of TJP.

Summary and Conclusions

In summary, our study for the first time showed that short-term starvation resulted in the downregulation of claudin-3 and 4 via dynamin-dependent but clathrin and caveolae-independent endocytosis. Interestingly, prolonged starvation resulted in the re-synthesis of TJP. Therefore, the transient endocytosis inhibition may provide important insight to prevent the starvation-induced TJP disruption and preserve gut barrier function in vivo.

Acknowledgement

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