The Bowen-Conradi EMG1 Variant is a Hypomorphic Allele

Joel Hardy, Michael Charette

INTRODUCTION

Bowen-Conradi Syndrome (BCS) is a ribosomopathy (ribosome assembly disorder) present in the prairie Hutterite population. It is characterized by severe development delay leading to death in early childhood. Clinical care for individuals with BCS is primarily supportive. Therefore, further research needs to be conducted to better understand the disease and to propose treatment options.

Figure 1: A child affected by BCS exhibiting the physical features that are arise with this ribosomopathy.

AIM

The aim of this project is to test whether the BCS variant (D86G in humans and D90G in yeast) of the ribosome assembly protein Emg1 is hypomorphic. In such situations a sequence variant causes structural changes in the protein that result in decreased protein abundance, loss of protein structure, and function. Here we assess the levels of the wild-type and BCS variant EMG1 protein along with probing for structural changes in the protein.

METHOD

Using a yeast model of BCS, we assessed the levels of FLAG tagged wild-type and BCS variant EMG1 by western analysis. Structure probing was done by partial trypsin digestion and western blotting.

RESULTS

- We observe a decrease in the protein levels of BCS variant EMG1 compared to wild-type for cells grown at 30℃ and 35℃. However, cells grown at 22℃ show more normal protein levels of BCS variant EMG1. This suggests a temperature dependent instability of the BCS variant.

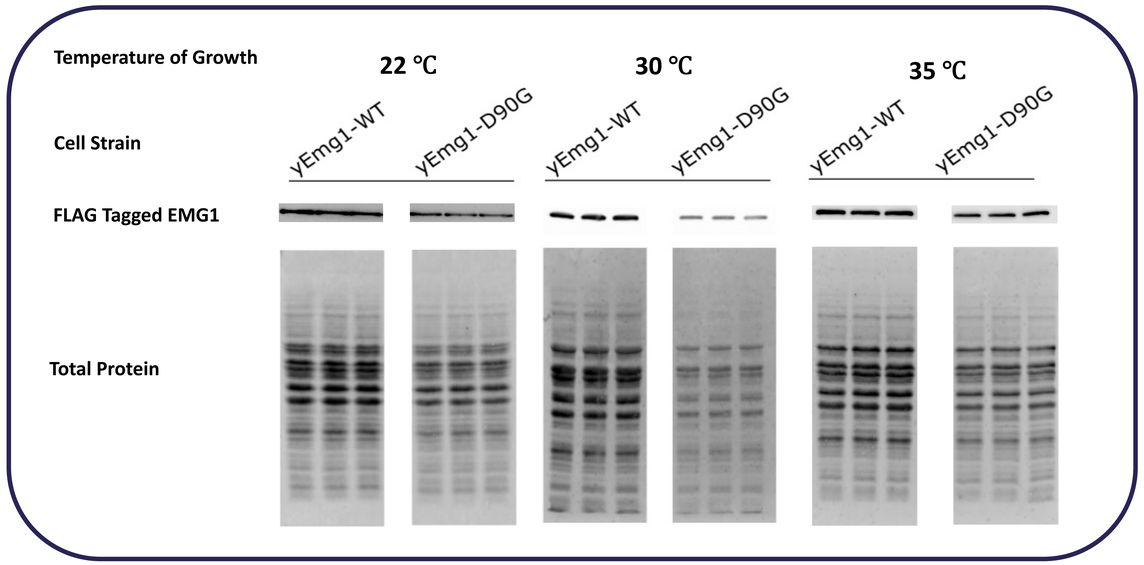
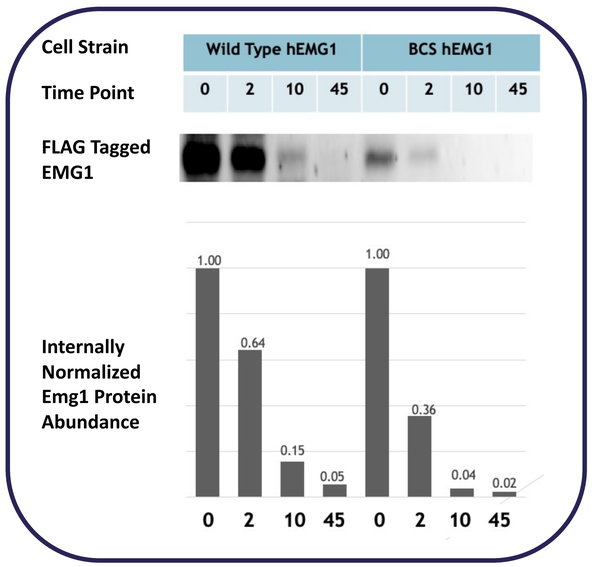


Figure 2 (top): In the experiment, cell strains containing WT EMG1 or D90G yEMG1 were grown at different 3 different temperatures. Equal amounts of cells were then harvested. For each sample equal volumes of prepared lysate was then ran through SDS-PAGE, in triplicate, and subsequently blotted for FLAG epitope after Western transfer.

Figure 3 (right): In the experiment, cell lysate from WT hEMG1 and D86G hEMG1 were collected. Trypsin enzyme was then added to the lysate and allowed to incubate at 25 ℃. At set time points, in minutes, aliquots of lysate was removed from each sample and boiled to stop the reaction. The samples were then loaded into an SDS-PAGE and subsequently blotted for FLAG epitope after Western transfer.



- In a trypsin sensitivity assay, we observe that wild-type EMG1 is relatively stable whereas BCS variant EMG1 is degraded more rapidly. A difference in Trypsin sensitivity in human Emg1 between the wild-type and BCS variants implies a structural change.

CONCLUSION

From our data, we conclude that BCS is due in part to local or global protein unfolding of EMG1 which decreases the protein's stability and abundance. Further studies will examine the ubiquitination levels of the wild-type and BCS variant EMG1.

Contact:

Joel Hardy

HARDYJW56@brandon.ca

Michael Charette

charettem@brandonu.ca

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