Curr Synthetic Sys Biol 2017, 5:2 (Suppl)
DOI: 10.4172/2332-0737-C1-012

4th World Conference on
SYNTHETIC BIOLOGY AND GENETIC ENGINEERING
November 09-10, 2017 Singapore

Polymerization is required for UNC 45A destabilization of microtubules in cancer cells

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Results from our laboratory have recently shown that UNC 45A is a cytoskeletal associated protein overexpressed in paclitaxel chemotherapy resistant cancer cells. Furthermore, we have shown that UNC 45A directly binds to microtubules (MTs) and destabilizes them. Our working hypothesis is that UNC 45A overexpression in paclitaxel resistant cancer cells augments destabilization of MTs and therefore, antagonizes paclitaxel effect on cancer cells. Recent data obtained in lower organisms (*C. elegans*) suggest that UNC 45A polymerization is required for its function, which may be required for its binding to microtubules. Thus, we aim to answer the question: Is polymerization of the mammalian UNC 45A required for its function? To do so we determine whether the human UNC 45A forms polymers *in vivo* (cancer cells) and *in vitro* (recombinant protein) and identify the domain of UNC 45A that is required for polymerization. To determine whether UNC 45A forms polymers; we treated cancer cells using disuccinimidyl suberate (DSS) crosslinking agent. We then confirmed polymer formation via Western blot. Our western blot shows that UNC 45A does indeed form polymers in human cancer cell lines. To determine which region of UNC 45A could be responsible for polymerization, we used a recombinant protein system of N and C terminal deletions of the UNC 45A gene. Full length, C terminal and N terminal truncated UNC 45A proteins were expressed in bacteria and purified. Protein expression was confirmed by Western blot analysis. Western blots of recombinant protein treated with DSS show that full length, deleted C terminal and deleted N terminal UNC 45A all form polymers. Further research will allow us to determine how domains of UNC 45A interact during polymer formation and if the neck region on UNC 45A is quintessential to polymerization.

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