Effects of Increased Nuclear Localization of Tip60 in Cancer Cells

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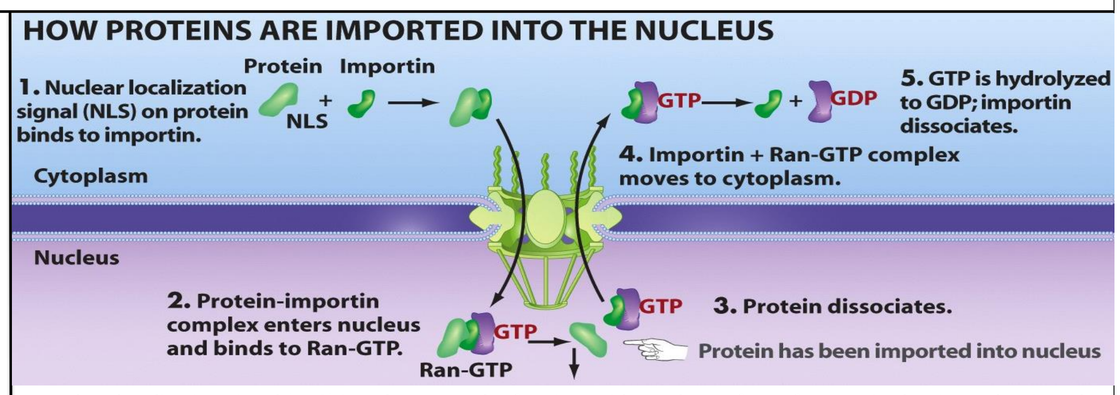
ABSTRACT

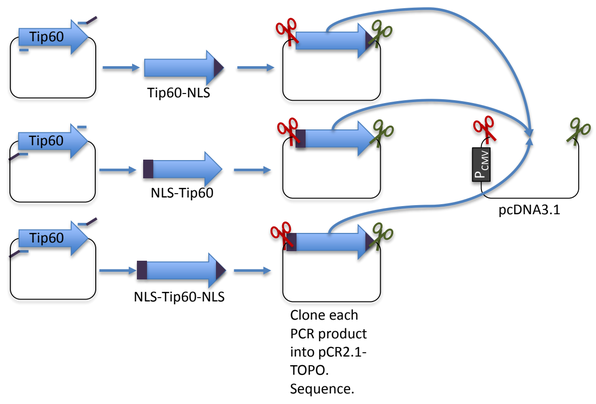
Tip60C is a 13-protein complex involved in transcription, DNA repair, and cell cycle regulation. In most cancers, the complex has been shown to be a tumor suppressor. Its catalytic subunit is the Tip60 protein, which is a lysine acetyltransferase responsible for acetylating targets including histones H3 and H4, transcription factor NF-κB, ATM kinase, and p53 tumor suppressor. We had previously shown that breast, lung, and pancreatic cancer cells treated with the chemotherapeutic drug paclitaxel and overexpressing Tip60 showed small but significant reductions in proliferation compared to paclitaxel treatment alone. We investigated whether the subcellular localization of Tip60 was necessary for its anti-proliferative activity. We added the prototypical nucleoplasmin bipartite nuclear localization signal to the amino-, carboxyl- and both termini of the Tip60 protein and analyzed the effects of overexpressing these proteins in breast and lung cancer cell lines. Using immunofluorescence we determined the localization of Tip60 and measured cell proliferation as compared to wild-type Tip60 (WT Tip60). While the addition of any NLS significantly increased nuclear localization, the C-terminal NLS had a larger effect than the N-terminal NLS. Although we increased nuclear localization, we did not observe any decreases in cancer cell proliferation with the NLS constructs compared to WT Tip60 in cells treated with paclitaxel. These results suggest that Tip60's anti-proliferative activity in cancer cells does not necessarily take place in the nucleus. We are continuing to investigate the molecular effects of increasing the amount of Tip60 in the nucleus.



A nuclear localization signal (NLS) is a short stretch of amino acids on the surface of a protein that is predominantly positively charged. We know that Tip60 gets into the nucleus. It may do this on its own through an endogenous NLS or as part of the Tip60C complex. Our aim was to get more Tip60 into the nucleus by the addition of an extra NLS.

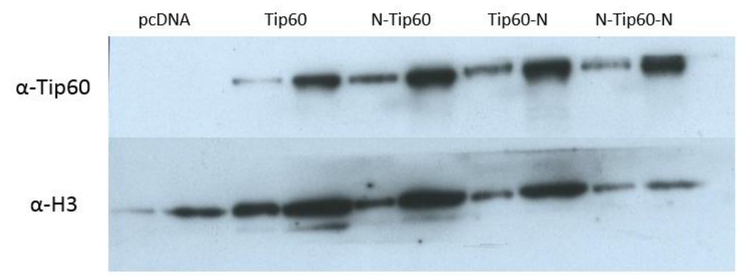
How do we increase nuclear localization of Tip60 in cancer cells?

Tip60 was amplified from a plasmid primers containing the NLS using PCR. Those PCR products were cloned into the pCR2.1-TOPO vector. Then they were cut out from there and ligated into the mammalian expression vector pcDNA3.1 to create the vectors for transfection.



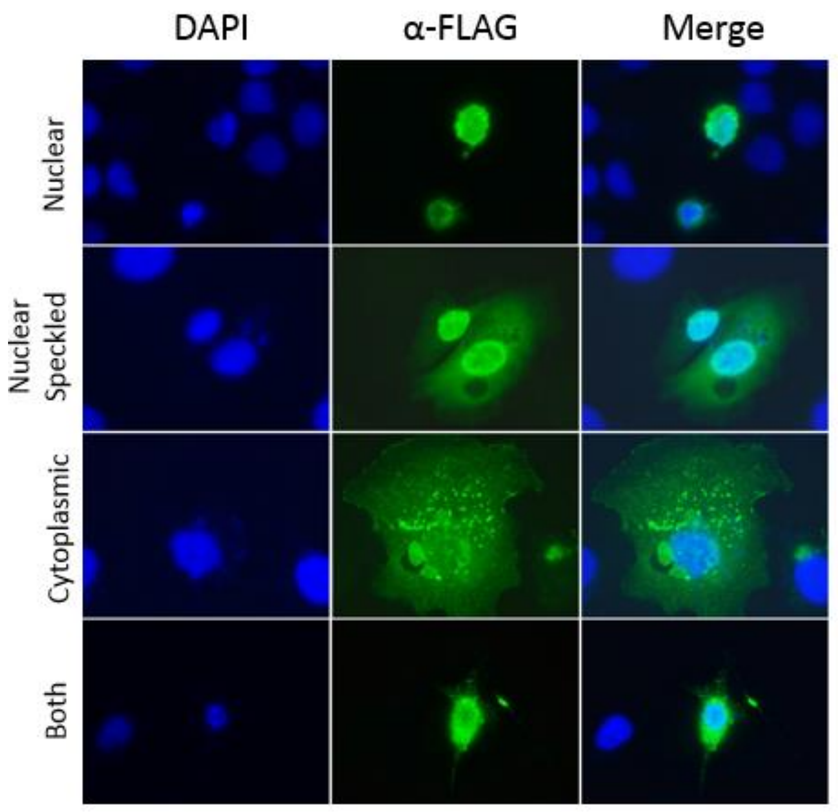
Does the NLS affect Tip60 expression?

HEK-293 cells were transfected with the indicated plasmids. 48 hours later whole cell extracts were made and subjected to Western blotting analysis with anti-Tip60 and anti-H3 antibodies.



The NLS does not reduce Tip60 expression.

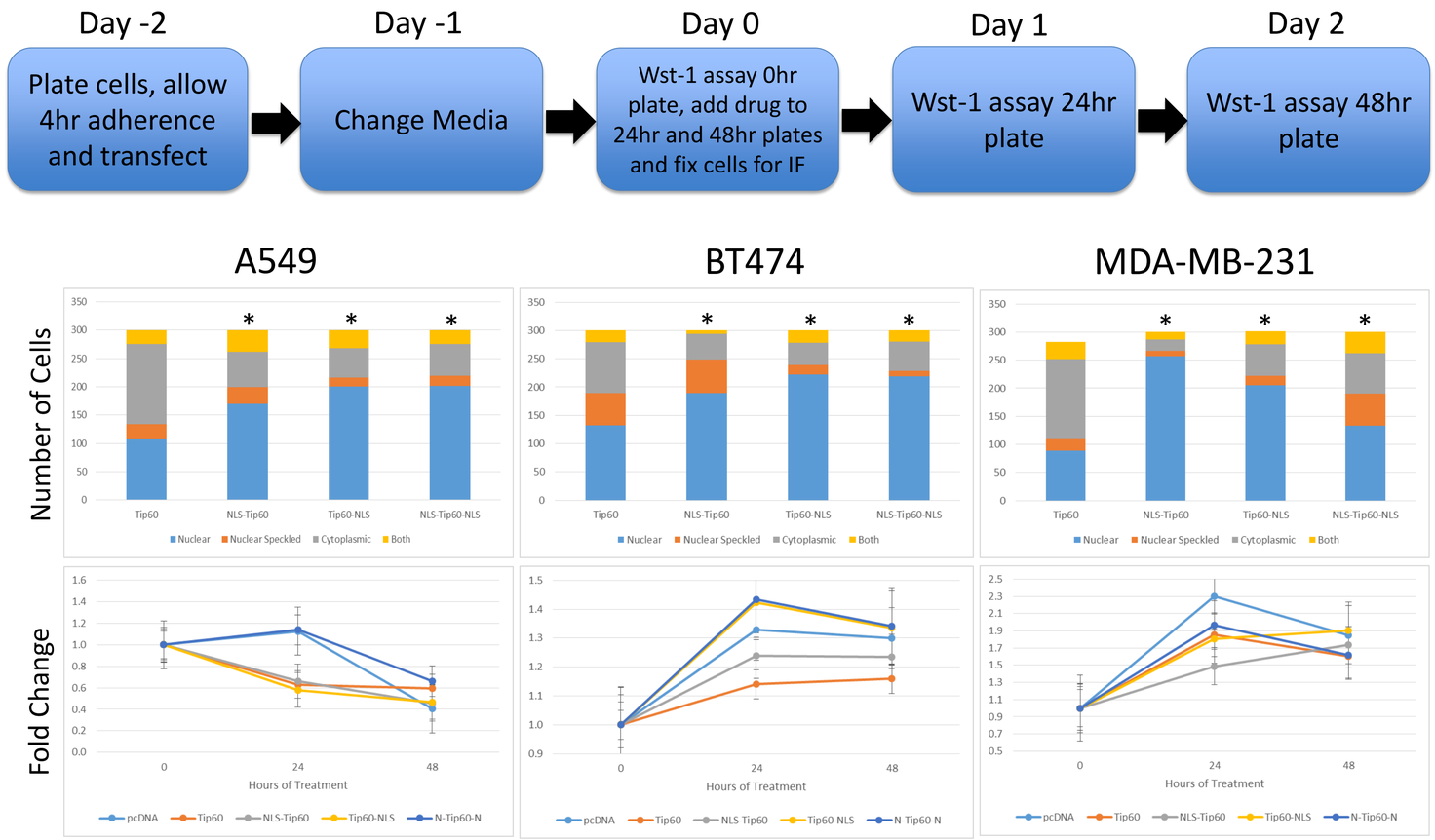
What types of localization of Tip60 were observed?



Cells were transfected with the different Tip60 plasmids then fixed with paraformaldehyde after 48 hours. The cells were then stained using α-FLAG antibodies and DAPI, a nuclear staining reagent.

We observed four different types of nuclear localization including nuclear, nuclear speckled cytoplasmic and both (nuclear and cytoplasmic).

Do changes in nuclear localization affect cell viability?



Increased nuclear localization of Tip60 (* $p<0.001$ by χ^2 test) does not appear have an effect on cell viability in the lung and breast cancer lines.

Conclusions

- Addition of a C-terminal NLS significantly increases Tip60 nuclear localization.
- Increased nuclear localization did not lead to reduced proliferation of breast and lung cancer cells.
- Perhaps Tip60's tumor suppressor activity takes place in the cytoplasm.
- Perhaps the extra Tip60 is not active.

Future Directions

- Does increased Tip60 nuclear localization lead to increased acetylation of nuclear targets (histones)?
- Does Tip60 overexpression lead to increased p53 acetylation?
- How much of the overexpressed Tip60 is incorporated into the Tip60C complex?

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