The Tumor Suppressor Protein RASSF1A modulates transcriptional activation of NF-AT

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ABSTRACT

Epigenetic silencing of the tumor suppressor protein RASSF1A has been described in numerous cancers, and many recent studies suggest utilizing methylation status of this gene as a prognostic marker. Several roles have been attributed to this protein including regulation of cell cycle progression, microtubule stability, DNA damage control, control of inflammation and modulation of apoptotic pathways downstream of death receptors. Here, we describe a novel role for this protein in the transcription regulation of the transcription factor Nuclear Regulator of Activated T Cells (NFAT) upon T cell engagement. Through cellular and molecular engineering, we see increased expression of RASSF1A in a human T cell line enhances transcriptional activation of NFAT when compared to vector-transfected control cells. Furthermore, co-transfection of RASSF1A with the NFAT regulator, calcineurin, resulted in a synergistic effect on NFAT transcription, suggesting that RASSF1A and calcineurin cooperate to induce maximal activation of NFAT.

DNA

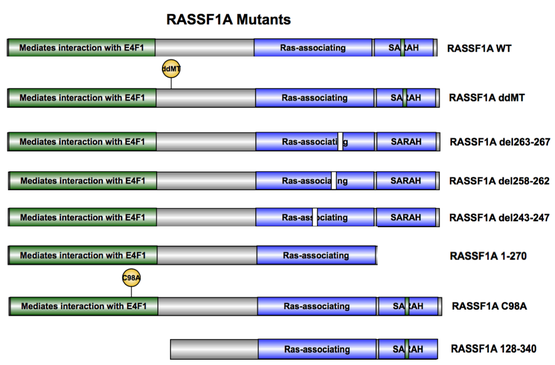


Figure 1: Locations of Mutation in RASSF1A

POINT MUTATIONS:
-ddMT131
-C98A

DELETIONS:
-263-267 -258-262 -243-247
-1-270 -128-340

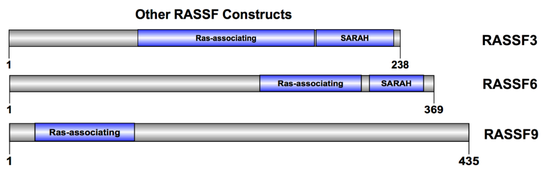


Figure 2: Structures of other RASSF Constructs

REGIONS

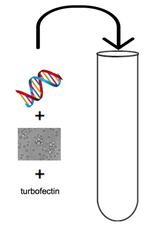
Ras- associating: Characteristic in Ras family proteins. Ras proteins cycle between inactive GDP-bound and active GTP-bound forms[4] and regulate a diverse portfolio of functions including cell proliferation and differentiation[1].

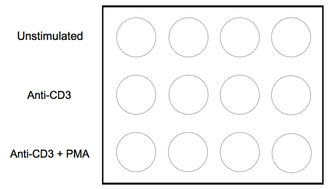
SARAH: found in C terminus of some eukaryotic tumor suppressors, mediates signal transduction[5]

METHOD

1. Transfection

Jurkat Cells were transfected with DNA using turbofectin. RASSF1A is has provided consistently positive results proving its activation of NFAT. The reason we use mutants is to see what specific interactions of RASSF1A correlates to the activation, by removing certain parts and seeing if stimulation continues.





2. Stimulation

36 hours after transfection, cells were either left unstimulated or stimulated with immobilized anti-CD3 antibody or anti-CD3 antibody plus PMA for 5 hours.

3. Luciferase Reporter Assays

Cells harvested and lysed with Passive Lysis Buffer and luciferase activity measured in luminometer, which measures light emission from samples. The reaction below is one that takes place in fireflies and is very energetically efficient, meaning the energy put in is directly converted into light. Since the DNA is fused to the luciferase reporter gene, the luciferase activity can be directly correlated with the activity of DNA. This is how we measured the activation of NFAT in the Jurkat cells.

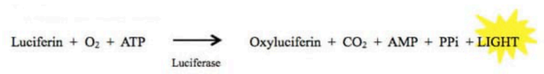


Figure 5: The reaction that occur in fireflies that allows them to emit light through the conversion of luciferin to oxyluciferin
https://bitesizebio.com/10774/the-luciferase-reporter-assay-how-it-works/

RESULTS

For the results, I took the data from the firefly luciferase cells and divided it by the values taken of the unstimulated Renilla cells. This allows me to normalize the numbers and make sure none of the elevated values are attributed to just a higher number of cells rather than actual NFAT activation.

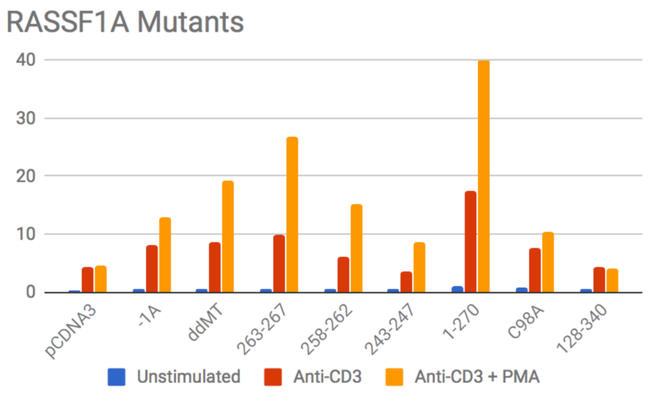


Figure 6: Graph of NFAT Activation in RASSF1A Mutants

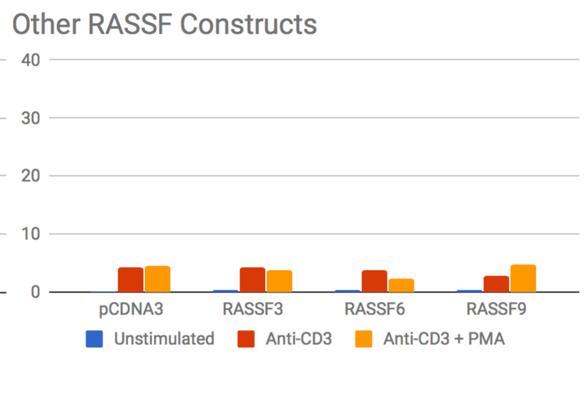


Figure 7: Graph of NFAT Activation in other RASSF Constructs

CONCLUSIONS

As we can clearly see form Figure 1, we see that there is definite enhancement of NFAT in RASSF1A compared to the pCDNA3, the vector. Interestingly, we also observe that there are a few mutations that seem to further enhance NFAT activation, the ddMT, 263-267, and 1-270. The mutations 258-262, 243-247, and C98A, though we do see some enhancement in comparison to the vector, it is still less than that of RASSF1A, which suggests that these mutants are in some way, preventing RASSF1A from carrying out its normal function. The 128-340 mutation actually seemed to have lower NFAT activation than even the vector, which was surprising. The ddMT disables interaction with microtubules, so no drop in activation means the activation of NFAT is independent of that. We also see that MOAP1-binding is not important in this case because the 1-270, which lacks that region, has high stimulation.

Figure 2 shows NFAT activations very close to that of the vector. Though RASSF3, RASSF6, and RASSF9 are known to be tumor suppressor proteins1, these findings show no correlation between these unmutated constructs and NFAT activation, meaning they probably obtain their tumor suppressing properties in other ways.

Similar to what we see in the 128-240 mutation that showed lower activation in Figure 1, these other RASSF constructs are also missing a diacylglycerol binding domain (C1/DAG). RASSF3 in particular bears strong resemblance to the 128-340 mutation, and also they seem to have fairly similar results. Both have lower activation than the vector and also have less NFAT activation in the CD3+PMA stimulation than the CD3 stimulation alone.

FUTURE RESEARCH

I would like to go on from here and look at RASSF5(NORE1). It does have a DAG binding domain. Looking at that could potentially tell us if the presence of that C1/DAG has anything to do with inhibiting NFAT activation. Something else interesting would be to look at some mutations of the other RASSF family members. We observed in the RASSF1A that some mutations had higher activation than the wild type RASSF1A, so perhaps there is similar activity in other mutants. The 1-270 lacking the MOAP1-binding region has consistently high values, so that is something possibly worth looking into. There is also a physical association between NFAT and the protein phosphatase calcineurin, so I could also go on to see if RASSF1A enhances calcineurin as well, as it theoretically should.

ACKNOWLEDGEMENTS

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4. "InterPro." Ras-Associating (RA) Domain (IPR000159) < InterPro < EMBL-EBI, www.ebi.ac.uk/interpro/entry/IPR000159.
5. "InterPro." SARAH Domain (IPR011524) < InterPro < EMBL-EBI, www.ebi.ac.uk/interpro/entry/IPR011524.

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