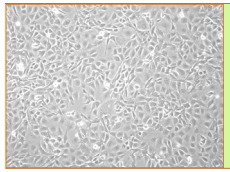

What's all the *fus* about?: *fus*as a potential cell-cycle regulator

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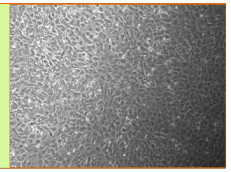
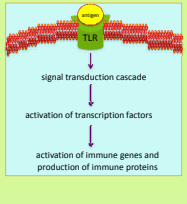
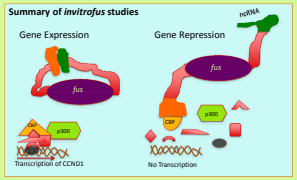


Introduction

This study was designed to explore the role of the *fus* protein in mouse lung fibroblast (MLF) homeostasis in response to innate immune stimuli.

In an innate immune response, cells recognize antigens and begin to produce cytokines—proteins that, among other functions, aid in the development of acquired immunity and activate lymphocytes and macrophages. The mechanism in which the cell recognizes antigens and alters its gene expression accordingly involves pathogen-associated molecular pattern recognition proteins called Toll-like receptors (TLRs) located both externally and internally in a variety of cells. There are many different TLRs associated with cells of the innate immune system, and each can be activated by a different pathogenic motif. An antigen binds to its corresponding TLR, thus activating a complex reaction cascade that results in the activation of various transcription factors that in turn activate and repress certain genes. (1).





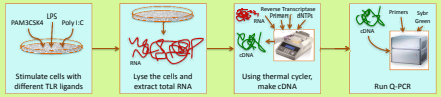
One protein that is known to be activated when stimulated via the TLR pathway is called *translocated in liposarcoma* (TLS), whose mouse homolog is called *fus*. TLS is an RNA-binding protein that regulates gene expression. A common cancer-causing mutation causes TLS to move to another part of the genome and fuse with another gene, thereby generating a fusion protein. One known target of TLS is the gene CCND1, which codes for cyclin D1 (2).

In this study, we aim to determine the downstream targets, function, and importance of the *fus* protein in MLFs.

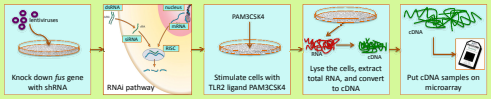
Methods

All experiments utilized mouse lung fibroblasts.

- Quantitative PCR (Q-PCR) was performed to measure the effects of different TLR ligands on immune response stimulation.



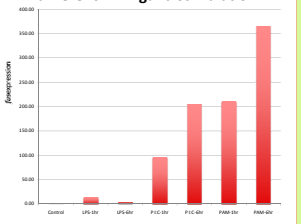
- *fus*shRNA constructs were introduced into MLFs to knockdown *fus*, and knockdown was confirmed by Q-PCR.
- Cells with *fus* knockdown were stimulated with TLR2 ligand PAM3CSK4 (PAM). Changes in gene expression in *fus*⁺ and *fus*knockdown cells were measured on Affymetrix microarrays, and data was analyzed using the computer program *Ingenuity*.



- Knockdown and control cells were arranged in a 96-well plate and imaged.

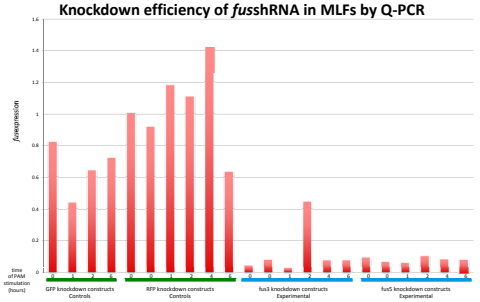
Results

fus fold induction in response to different TLR ligand stimulation



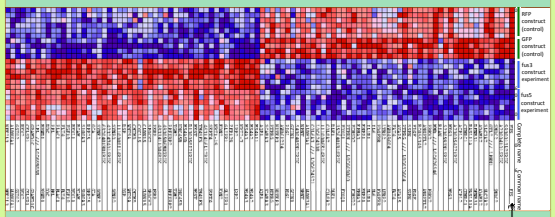
MLFs were stimulated with TLR2 ligand PAM, TLR4 ligand LPS, and TLR3 ligand Poly I:C for one and six hours each. RNA was isolated from each culture, converted to cDNA, and run on Q-PCR, testing expression of the GAPDH, TNF, Il-1b, Il-6, Il-12, Cxcl10, Cox2, and *fus*genes. For almost all genes, PAM induced the most gene expression relative to the unstimulated control. This graph shows relative levels of *fus* mRNA within the cell.

Knockdown efficiency of *fus*shRNA in MLFs by Q-PCR

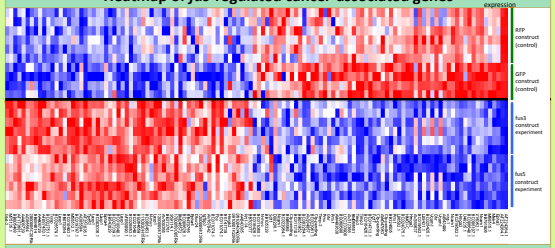


MLFs were infected with lentiviruses that introduced four different shRNA constructs into their genomes—two control constructs (GFP and RFP) and two experimental constructs (fus3 and fus5) that were designed to knock down the *fus* protein. These cell populations were stimulated with PAM at different time points—zero, one, two, four, and six hours. RNA was extracted from each culture, converted to cDNA, and then run on Q-PCR to test for *fus* mRNA levels within the cell.

Heatmap of the 50 most upregulated and downregulated genes

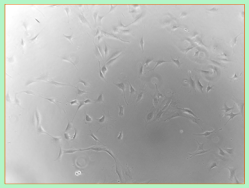


Heatmap of *fus*-regulated cancer-associated genes

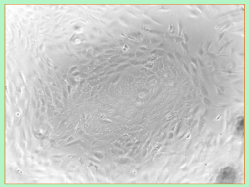


The samples in the above figures were assayed for gene expression using Affymetrix Microarrays. The heatmaps show relative levels of mRNA within the cell.

GFP control cells after 18 hours



fus3 knockdown cells after 18 hours



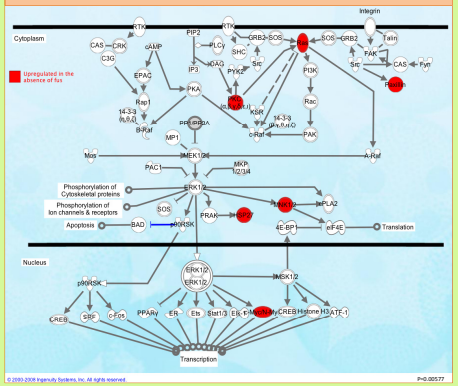
Cells with fus and control knockdown constructs were plated with equal cell concentrations in a 96-well plate and left to incubate for 18 hours. These pictures indicate that there may be differences in proliferation rates between the two populations.

*An experiment in which knockdown and control cells are quantified, plated at equal number, left to incubate, and then quantified again has not yet been done.

Conclusions

- *fus* regulates a large and diverse group of genes, most of which are tissue growth-related and cancer-related genes.
- Absence of the *fus* protein leads to upregulation of known oncoproteinsRas, Paxillin, PKC, MNK, HSP27, and Myc.
- These proteins are all involved in activating the extracellular signal-related kinase/mitogen-activated protein kinase (ERK/MAPK) Signaling Pathway (shown below, with upregulated proteins shown in red), a pathway often implicated in cancer.

ERK/MAPK Signaling Pathway



- The ERK/MAPK signaling pathway is involved in regulating cell proliferation and apoptosis. When this pathway is inappropriately activated, apoptosis will not occur when it is supposed to, and cells will proliferate when they are not supposed to. This can lead to cancer.
- A comparison of *fus* knockdown and control cells by microscopy indicates that *fus* knockdown may affect cell proliferation rates.
- **We hypothesize that *fus* acts as a tumor suppressor protein via repression of oncogene expression, and that *fus*(or TLS) translocation and fusion results in overactive ERK/MAPK activity.**

Future Experiments

- Immunoprecipitation experiment in which *fus* is pulled down and all proteins and nucleic acids associated with it are identified and investigated.
- *fus* knockdown *in vivo* and *in vitro* in different cell types to determine its functional effects in more detail.

Acknowledgements

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References

1. Gregory M. Barton and RuslanMedzhitov. (2003). *Linking Toll-like receptors to IFN-α/β expression*. Nature Immunology. Vol. 4: 432-433.
2. Xianting Wang *et al.* (2008). *Induced ncRNAsallosterically modify RNA-binding proteins* in cis *to inhibit transcription*. Nature. Vol. 454: 126-130.