

Investigating the consequence of RNA editing on the function of the dsRNA sensors.

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The aim of this project was to identify highly immunogenic dsRNA that are that are capable of binding to the dsRNA sensors and activating an immune response. To achieve this objective, we fused the dsRNA sensors MDA5 and PKR to the deaminase domain of ADAR2. Both PRK and MDA5 will bind to dsRNA and these dsRNAs will be identified by undergoing editing by ADAR2. This editing can easily be identified as there will be a transition from A to G in RNA Seq analysis.

To achieve this, we generated constructs encoding protein fusions between the deaminase domain of ADAR2 and the dsRNA sensors MDA5 and PKR. Stable cell lines expressing these fusion proteins under doxycycline promotor were generated so the level of induction of the fusion proteins could be tightly regulated. We have performed RNA Seq but have not yet completed the analysis. We will identify the dsRNAs that bind PKR and MDA5 as they will have an increased A to G signature. Analysis of these dsRNAs will reveal if the same dsRNAs bind preferable to both PKR and MDA5 and if the sensors have the same sensitivity to endogenous dsRNA levels.