430 SMART SENSOR PROMOTERS DRIVE STATE-SPECIFIC GENE CIRCUITS TO CONVERT IMMUNOSUPPRESSIVE MACROPHAGES INTO AN ANTI-TUMOR PHENOTYPE

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Background Immunosuppressive signaling from myeloid cells and macrophages in tumor microenvironments hampers the clinical efficacy of immunotherapies. Macrophages, however, are a highly plastic cell type that can be polarized to either a pro-inflammatory (M1) or an anti-inflammatory (M2) state. Macrophages promote tumor growth and immune suppression in an M2 state but have anti-tumor activity in an M1 state. We have built gene circuits comprising promoters specific to the M2 state that drive controlled expression of immune payloads that polarize cells towards the M1 state. These gene circuits enable macrophages to sense immunosuppressive cues such as those in the tumor microenvironment and respond by enacting an inflammatory anti-tumor response.

Methods We performed ATAC-Seq on M2-polarized primary human macrophages and identified regions of preferentially open chromatin in the M2 state relative to M0 and M1 states as putative M2 state-specific enhancers. We then paired those enhancers with a panel of minimal or core promoters to create M2-state specific promoter candidates. M2-state specific promoters were used to express an M1-associated payload (e.g. IFN-gamma) to create a 'phenotype switch' where the macrophages could switch from an M2 polarization state to an M1 phenotype. Macrophage phenotype was assessed using multi-color flow cytometry and cytokine expression.

Results When paired with a single minimal promoter, enhancer-based promoters were selective with >100-fold specificity for the M2 state, but weak with <15% of the strength of EFS. By swapping out the core promoter, we increased promoter strength >10-fold while maintaining M2 state selectivity. Our M2-state specific promoters were then used to drive IFN-gamma expression or other similar pro-inflammatory signals to create a phenotype switch. M2 polarization of these transduced macrophages induced these phenotype switch circuits to turn on and transpolarize the cells towards a pro-inflammatory phenotype despite being polarized to an opposing state. The M2-polarized macrophages also secreted high levels of pro-inflammatory cytokines indicative of their switch towards an M1 phenotype.

Conclusions Immunosuppressive signaling by tumor associated macrophages and myeloid derived suppressor cells limits the efficacy of cancer immunotherapies. Abrogating this pathological signaling can restore anti-tumor immune responses. Here, we leveraged native immunosuppressive signals present in tumor microenvironments to turn on expression of inflammatory, anti-tumor payloads in macrophages. Our 'sense' and 'respond' gene circuits in macrophages can be used to activate an inflammatory anti-tumor immune response for applications such as adoptive cell therapies or gene therapies.

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