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Antineoplastic targeted therapy: Identifying and manipulating cancer associated fibroblasts (CAFs) with aberrant mechanotransduction

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The tumor microenvironment (TME), composed of resident and infiltrating stromal cells, plays a critical role in supporting tumor progression, dissemination, and therapeutic resistance. Among these stromal components, cancer-associated fibroblasts (CAFs) are pivotal in shaping the TME and influencing the behavior of both cancer and stromal cells. Preclinical studies have shown that non-specific targeting or depletion of stromal fibroblasts in the TME can paradoxically enhance tumor aggressiveness. This underscores the need for highly specific therapeutic approaches to target CAFs while minimizing associated toxicity. Despite extensive research, no single specific CAF marker has been identified, hindering the development of CAF-targeted therapies. Biomarkers for aggressive CAFs remain elusive and their identification relies heavily on functional characterization.

We hypothesize that aggressive CAFs, particularly those exhibiting elevated mechanotransduction activity, undergo distinct phenotypic changes that promote the expression of specific cell membrane epitopes. These epitopes could serve as biomarkers for identifying and modulating aggressive CAFs. In our laboratory, we have postulated that YAP activation is a key feature of certain aggressive, mechanically activated CAF subsets (myCAFs). YAP, a transcriptional co-activator in the Hippo pathway, plays a critical role in the transition from quiescent fibroblasts to CAFs. Its function is essential for promoting matrix stiffening, cancer cell invasion, and angiogenesis.

We further hypothesize that YAP regulates the expression of genes encoding membrane-bound epitopes specific to aggressive CAFs, or modulates their post-translational modifications, thereby altering their 3D structure. The characterization of these proteins will enable the identification and specific manipulation of these set of CAFs. To explore this, we are utilizing combinatorial chemistry approaches, including SELEX (Systematic Evolution of Ligands by Exponential Enrichment), to identify aptamers that bind specifically to these epitopes. Using murine and patient-derived CAFs, we aim to generate aptamers that selectively bind to aggressive CAFs with high YAP activity.

In parallel, we have engineered a murine CAF line in which YAP can be selectively degraded through rapid and effective degron tag binding. Preliminary results indicate that YAP degradation reduces YAP function, as confirmed by Western blot and luciferase assays. The next step involves assessing the biological consequences of YAP downregulation in this model, with the goal of identifying aptamers that bind specifically to wild-type CAFs (high YAP) but not to YAP-depleted CAFs (low YAP).

Our data suggest that generating an edited CAF line with reduced YAP activity provides a valuable control for distinguishing aggressive, YAP-high CAFs, from their edited less aggressive counterparts. This strategy could be particularly relevant for selectively targeting aggressive, pro-tumorigenic CAFs. Ultimately, if we identify an aptamer that binds to a CAF-specific epitope, it could be functionalized with lipid-polymer nanoparticles (LPNPs) for targeted drug delivery.

To this purpose, we aim to use aptamer-based technology to identify a YAP-specific aptamer and subsequently design a YAP-proteolysis-targeted degrader (YAP-PROTAC) to be incapsulated in CAF-specific LPNPs. We posit this may represent a novel and effective approach to lead to target YAP and potentially reverse or reprogram aggressive CAFs into anti-tumor CAFs or NFs. Altogether, this strategy could present a new and attractive method to minimize off-target toxicity while specifically modulating and identifying CAFs, ultimately improving cancer

prognosis.