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This project focuses upon Diffuse Gastric Cancer (DGC), a frequently lethal cancer, marked by its characteristic growth patterns with lack of cellular cohesion, highly invasive spread and marked propensity for metastasis. This proposal builds upon new progress in the study of DGC, bringing together a collaborative team of investigators with provocative new findings regarding the role of RHOA in the pathogenesis of this disease and several newly developed mouse model systems. These data and resources bring new opportunities to substantively advance the study of these deadly and understudied cancers. A first set of data underlying this application followed our recent identification of a novel stem cell population in gastric glands marked by *Mist1* expression (Yokoyama et al, *Cancer Cell* 2015). These cells were demonstrated to give rise to DGC following engineered loss of tumor suppressor *Cdh1*. However, development of DGC required the secretion of *Wnt5a* by cells in the stem cell niche, with *Wnt5a* acting by activating GTPase RhoA in the *Cdh1*- null gastric cells. In parallel, we made a set of novel genomic discoveries, finding that ~20- 30% of DGCs harbor genomic aberrations impacting RHOA, either highly recurrent missense mutations of RHOA or a recurrent fusion gene including *ARHGAP26*, a RHOA regulator (TCGA, *Nature*, 2014). In this context, delineating the functions of RhoA in DGC pathogenesis, spanning both the role of Wild-type RHOA following *Cdh1* loss and the oncogenic functions of RHOA mutations, emerge as critical paths towards the identification of therapeutic targets and understanding of basic pathophysiology of DGC formation. In our first Aim, we evaluate activation of wild-type RHOA in normal gastric corpus stem cells, and in early progression of *Cdh1*- deficient diffuse gastric cancer. We propose to test our hypothesis that RHOA is a mediator of *Wnt5a* effects upon corpus stem cells, especially following *Cdh1* loss. These results will have immediate relevance to the definition of mechanisms of DGC initiation, clearly informing efforts to prevent and treat these deadly cancers. Our second aim evaluates RHOA somatic mutations in the initiation and progression of diffuse gastric cancer. In this aim we further characterize the biochemical and phenotypic effects of highly recurrent missense mutations of the RHOA GTPase identified in DGC. We will also functionally validate which RHOA effectors are essential for oncogenic activity of these mutants in both in vitro and in vivo systems, including our novel DGC mouse model driven by *Cdh1* loss and RhoA mutation. Through these studies we hope to determine mechanisms of RHOA mediated transformation and identify specific pathways that are critical to the pathogenesis of DGC, findings with immediate potential relevance to the development of new therapeutic targets.