Crenolanib inhibition of PDGFRα+ fibroblast-like cells in visceral smooth muscle

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Aims

The purpose of this study is to disrupt PDGFRα signaling in gastro-intestinal smooth muscle using a potent new inhibitor of PDGFRα, Crenolanib.

Abstract

Platelet derived growth factors (PDGFs) and their receptors (PDGFRs) play important roles in cell proliferation, survival, and migration . The PDGF/PDGFR signaling pathway plays a crucial role in embryonic development and for interstitial and mesenchymal cell proliferation . Primary cancer cells in the GI with abnormal expression of receptor tyrosine kinases like c-Kit and PDGFRs have been implicated in the formation of gastro-intestinal stromal tumors (GIST).

Fibroblast-like cells (FLCs) exist in the smooth muscle of all visceral organs yet their functional role throughout the viscera is unclear . It has recently been demonstrated by studies within our group that these FLCs express PDGFRα . These studies demonstrated for the first time that PDGFRα+ cells are a novel class of excitable cells with the molecular and ionic apparatus to mediate enteric inhibitory responses to purines in GI muscles.

Using Crenolanib, a novel potent inhibitor of PDGFRα, we have disrupted PDGFRα expression and signaling in normal BALB/c mice. Crenolanib is being developed to treat cancers with populations of primary cells over-expressing PDGFRα including GIST. To track the specific changes in GI visceral smooth muscle cell populations and changes in post-junctional neural responses, we examined changes in protein abundance, gene expression, and used intracellular microelectrodes to examine electrophysiological properties and responses to stimuli. Given the intimate anatomical relationship between Kit+ ICCs and PDGFRα+ FLCs, their structural similarities, and their defining expression of receptor tyrosine kinases, changes in the dynamics of both of these cell populations during Crenolanib inhibition was monitored within this model. Age and species matched pairs of mice injected with either vehicle or Crenolanib were collected from postpartum days 10 through 25 and analyzed for differences in gross anatomy and weight, electrophysiology, PDGFRα protein distribution by western blot and immunofluorescence, and gene expression by both end-point PCR and qPCR. These analyses show a distinct decrease in the protein abundance and gene expression of PDGFRα in Crenolanib injected mice; inhibitory neurological signaling is also affected, with a highly significant decrease in purinergic inhibitory responses to stimulation, which indicates this as one of the functional roles of FLCs in the *Tunica muscularis*. Monitoring FLCs and ICCs in this inhibition model has provided valuable information on the endogenous cellular dynamics that exist between ICCs and FLCs; it also sheds light on their relationship during pathophysiolgical disorders, including the development of GISTs .

Design

1. Balb/c mice administered vehicle or Crenolanib (100µg/g body weight) from P1-P16.
2. Mice were euthanized at P13-16 and examined for gross morphological differences.
3. The complete GI was dissected and mucosa stripped.
4. Isolated smooth muscle from stomach, small intestine, and colon was examined for PDGFRα protein distribution (western blot, immuno-fluorescence and gene expression (qPCR)).
5. Smooth muscle was examined for electrophysiological properties, including slow wave analysis and responses to neurological stimulation

Results

- Crenolanib injected mice had decreased weight and length of complete GI, as well as areas of intestinal distension, in comparison to vehicle injected mice
- PDGFRα was decreased on the level of both protein abundance and gene expression in animals administered with Crenolanib
- qPCR analysis reveals a decrease in the expression of PDGFRα, SK3 (Kcnn3), as well as P2Ry1 (electrophysiologicaly significant), and an increased expression of apoptotic protease activating factor (APAF1).
- C-Kit and SMMHC immunofluorescence was unchanged, however SK3, a secondary marker of PDGFRα+ FLCs, was also decreased in Crenolanib injected animals, as seen by immunofluorescent co-labeling with PDGFRα
- Slow waves in all GI tissues were statistically unchanged, but purinergic responses to stimulation, a function of PDGFRα+ FLCs, were drastically decreased in all GI tissues

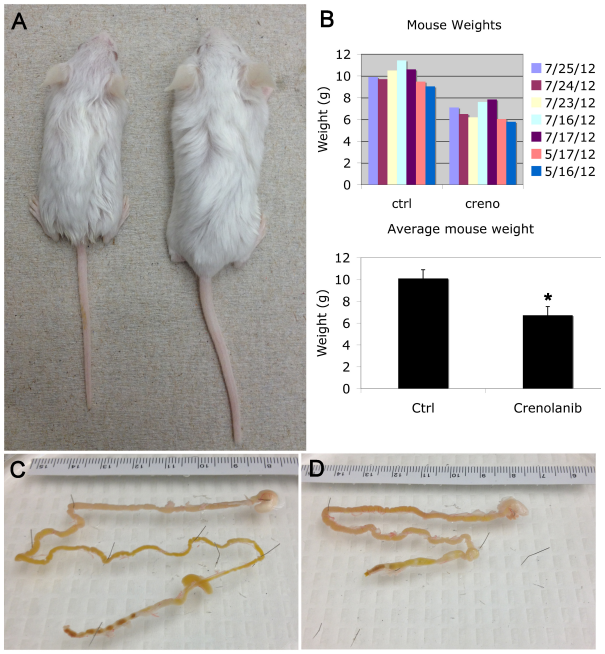

Figure 1. Size, weight, and total GI lengths

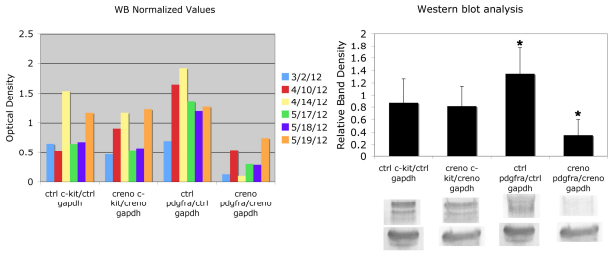

Figure 2. Western blot protein abundance analysis

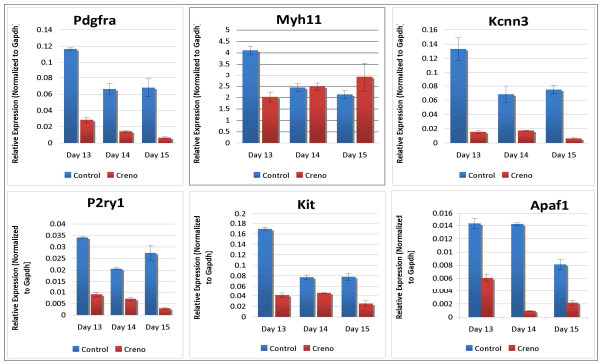

Figure 3. qPCR analysis of Control and Crenolanib treated small intestine smooth muscle

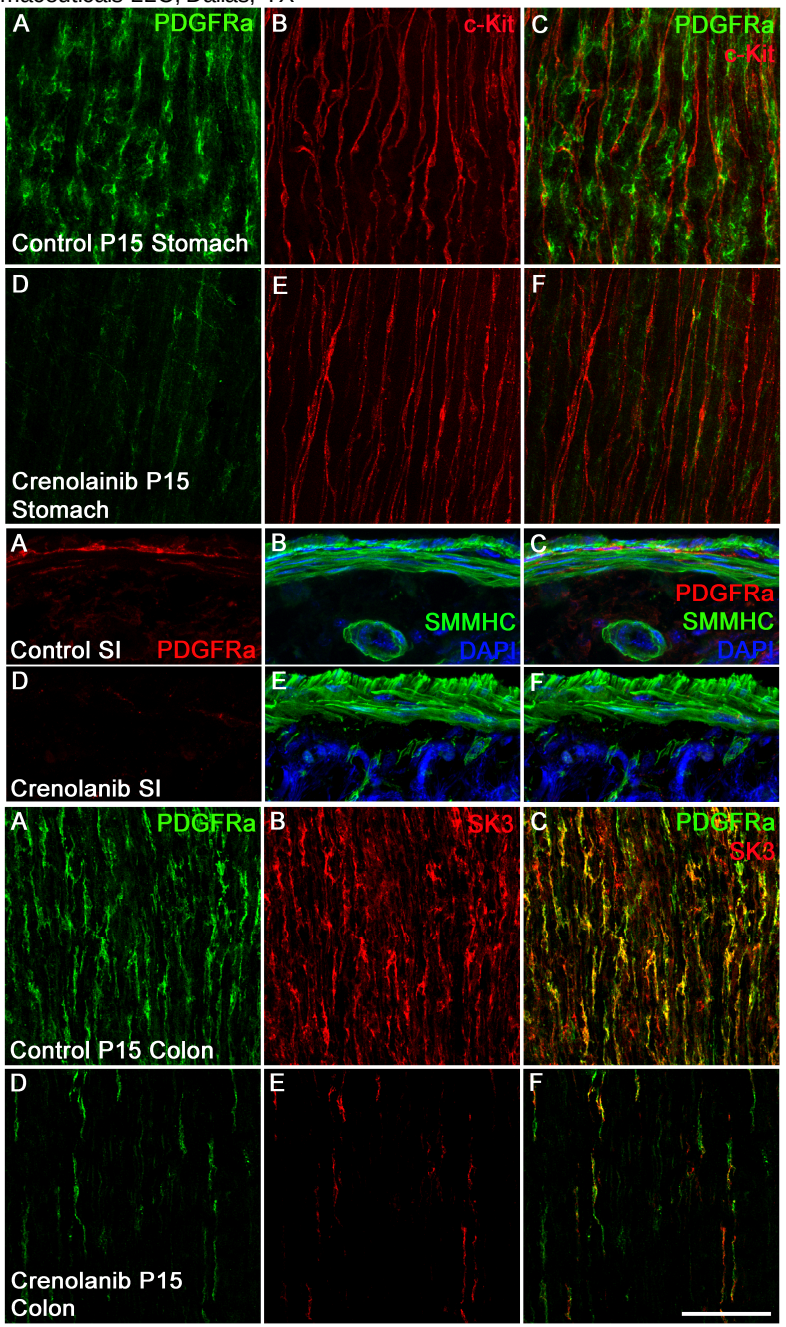

Figure 4. Immuno-fluorescent analysis of GI tissues. Crenolanib and control tissues: Whole mount stomach is shown with PDGFRα (A) and c-Kit (B). Cross sectional labeling of small intestine (SI) PDGFRα (A) and SMMHC (B). Whole mount of colon PDGFRα (A) and secondary marker SK3 (B). Merge of control (C) and Crenolanib treated (F) images.