Diabetic Complications Consortium

Application Title: Interstitial cells in diabetes disrupted gastric motor responses.

Principal Investigator: Sean M. Ward

1. Project Accomplishments:

The main achievement that was obtained from this Diabetic Complications Consortium (DiaComp) Pilot & Feasibility program proposal was the ability of the P.I. to use the funds supported by this Pilot and Feasibility grant to obtain significant NIH RO1 funding (NIH DK 057236).

In addition to this funding the P.I. and named investigators have made significant advances at studying cells that make up the **S**mooth muscle **I**nterstitial cells of Cajal and **P**DGFRα+ cells that make up the SIP syncytium in the human stomach and the changes that these cells undergo in type II diabetes. This advances will be discussed in detail under Specific Aims.

Specific Aims:

Aim 1: *Examine the structural relationship between motor nerves and PDGFRα$^+$ ICs in the human stomach and how this changes in the gastric fundus and antrums of patients with type II DM.*

Results: In order for an intestinal cell to act as a mediator between enteric nerve terminals and smooth muscle cells in the human stomach they must be closely associated with nerve fibers in this organ. We have previously shown that such a relationship exists in the human colon (Kurahashi et al., 2013) but were not sure that this was applicable to human stomach. We have spent considerable time performing dual label confocal microscopy to determine if PDGFRα+ cells are preferentially associated with enteric motor nerve terminals in human gastric muscles. **Figure 1** illustrates a typical example of a confocal image revealing close anatomical association between inhibitory nerves (neuronal nitric oxide synthase containing) and interstitial cells (ICC and PDGFRα+ cells in the circular muscle layer of the human gastric fundus and antrum. Close apposition between inhibitory nerve fibers and ICC and PDGFRα+ cells was observed for distances up to several hundred microns in both cell types and in both regions of the human stomach These data strongly suggest that the two interstitial cell classes (ICC and PDGFRα+ cells) may act as cellular intercelators in enteric gastric motor responses as observed in other animal species. We also further examined if there were changes in interstitial cells from patients with type II diabetes (DM). **Figure 2.** illustrates similar confocal images taken from the gastric fundus and corpus of a patient with type II DM. There was a marked reduction in both classes of interstitial cells from gastric tissues of this patient. There also appeared to be a disorganization between enteric nerves and ICC and PDGFRα+ cells. These data suggest that there is loss of ICC and PDGFRα+ cells and argues previously published data that there is no disruption or loss of

PDGFRα+ cells in human DM tissues (Grover et al., 2012). These data are currently being prepared for publication.

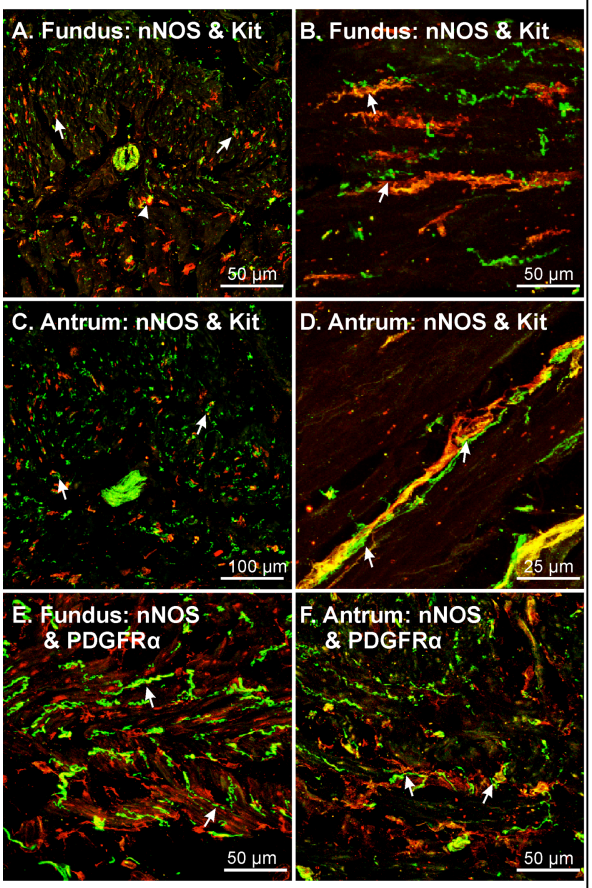

Fig. 1. Confocal immunohistochemistry results of proximal and distal stomach tissue in a non-diabetic control patient. A&B Fundus, C&D Antrum. nNOS [green] & Kit [red]. E. nNOS & PDGFRα [red] in fundus. F. nNOS & PDGFRα [red] in antrum. Note the close apposition between enteric nerves and ICC and PDGFRα+ cells (arrows).

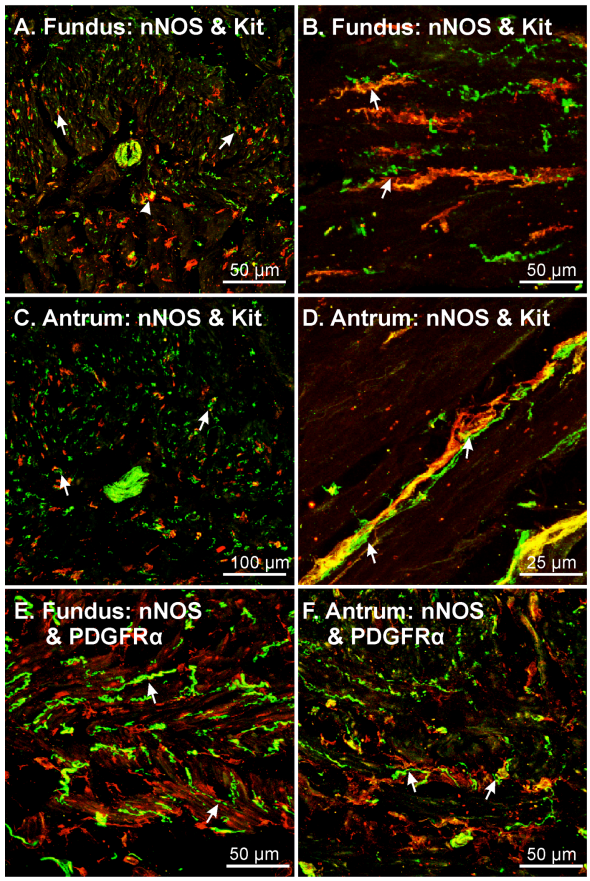

Fig. 2. Confocal immunohistochemistry results of proximal and distal stomach tissue in a DM patient. A&B Fundus, C&D Antrum. nNOS [green] & Kit [red]. E. nNOS & PDGFRα [red] in fundus. F. nNOS & PDGFRα [red] in antrum. Note the reduction in enteric nerves, ICC and PDGFRα+ cells. There is also a reduction in the close apposition between enteric nerves and ICC and PDGFRα cells (arrows).

Aim 2: *Determine the gene expression profiles of PDGFRα^{+} ICs in human stomach and the changes in expression profiles in gastric tissues from patients with diabetes (DM).*

Results: Over the funding period we have performed an examination to determine the expression changes in selected pacemakersome and neuroeffectorsome gene transcripts at the qPCR level in the proximal, mid and distal stomach of patients with type II DM compared to controls. There was a significant reduction in PDGFRα, Kit and Ano1 in the fundus and antrum compared to controls. These data support the protein reduction (observed at the immunohistochemical level) and disruption of PDGFRα+ cells and ICC in DM patients. The reduction in Kit is further

supported by a reduction in Ano1, which is the pacemaker current in ICC and is a selective marker for these cells in the stomach. There are also regional differences in the decrease in PDGFRα, Kit and Ano1 in the human stomach that has not been reported before. These data strongly support the hypothesis that there are changes in the **SIP** syncytium that require further evaluation (see below). There was also a significant reduction in nNOS or NOS1 in the human antrum but not in the fundus or corpus.

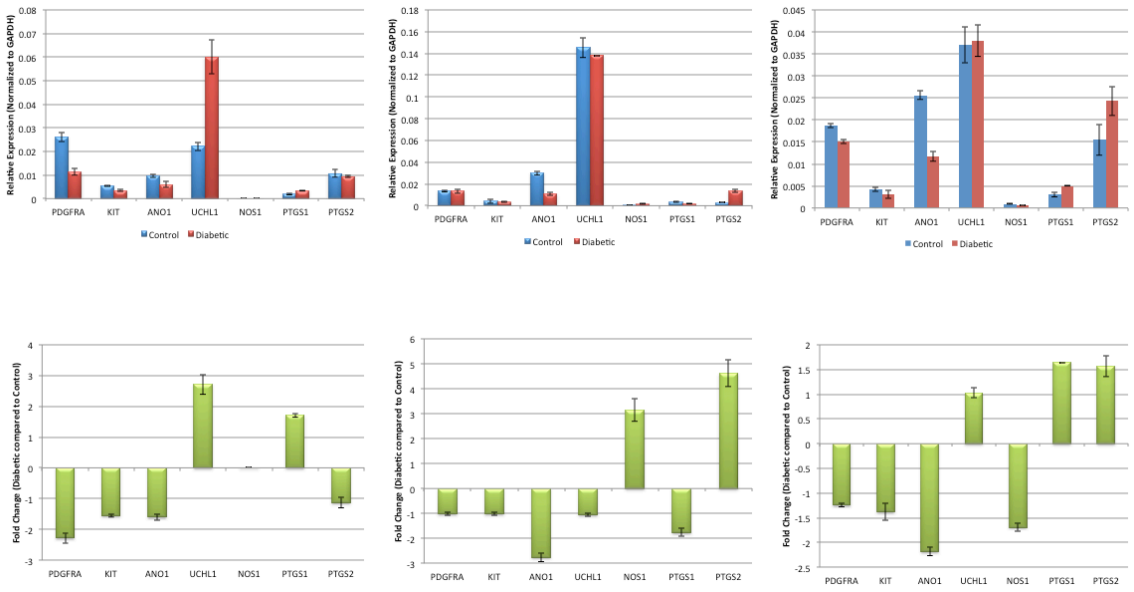

Fig. 3. Upper Panels: Relative expression of selected pacemakersome and neuroeffectorsome gene transcripts in the proximal, mid, and distal stomach of age matched diabetic (RED) and non-diabetic (BLUE) control patients. **Lower Panels:** Fold change in gene expression seen in selected pacemakersome and neuroeffectorsome transcripts in the proximal, mid, and distal stomach of a diabetic patient compared to a non-diabetic patient.

This last year we have also perfected the enzymatic dispersion and isolation of ICC an PDGFRα+ cells from human gastric tissues. **Figure 3** shows isolated PDGFRα+ cells and ICC from the human gastric antrum following enzymatic dispersion and immunolabeling with antibodies. Both PDGFRα+ cells and ICC were labeled with antibodies against external epitopes to their specific receptors (i.e. PDGFRα+ and Kit). Double labeling was performed to distinguish ICC from mast cells that are also present in this tissue.

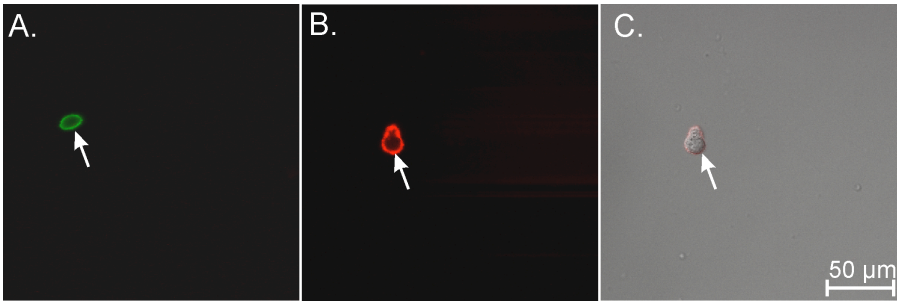

Fig. 4. Enzymatic dispersion of PDGFRα+ cells (A) and Kit+ ICC (B and C) from the human gastric antrum (arrows). Panel C shows a DIC image of the ICC in panel B. These cells can be used for FACS sorting and molecular analysis of individual SIP cell populations and gene expression analyses compared between isolated cells between control and DM tissues.

Enzymatic isolation of interstitial cells that make up the SIP syncytium has allowed us to purify larger numbers of specific cell populations using a fluorescent activated cell sorter (FACS) **Figure 5** shows an example of such a purification of PDGFRα+ cells from human gastric antrum. Similar purification has been performed for ICC. This is allowing us to collect sufficient cell numbers so that they can be interrogated to determine gene transcripts and the changes that occur in individual cell populations in DM tissues.

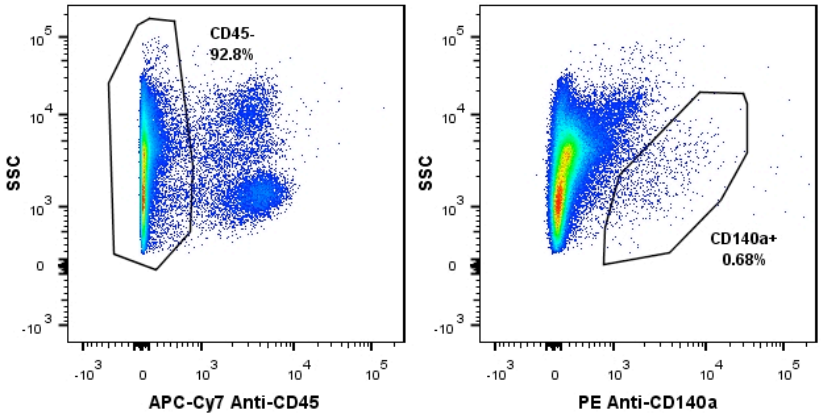

Fig. 5. Left Panel: Labeling of CD45+ cells to remove cells of hematopoietic origin including mast cells, macrophages and other neutrophils and dendritic cells. **Right Panel:** From the cells identified by the gate on left panel we further purified CD140a+ cells which represent PDGFRα+ cells. Similar experiments have been performed on human ICC populations from the gastric antrum.

Aim 3: Examine the functional consequences of the molecular remodeling of PDGFRα$^+$ cells in gastric tissues from patients with type II DM.

Results: We are continuing to perform a detailed analysis of pacemaker activity and post-junctional neuroeffector responses in gastric tissues from DM and non-DM muscles. Initially we had difficulties in performing functional studies due to the tissues but we have been successful in overcoming these and now able to obtain reliable results. We have seen differences in the frequency and amplitude of slow waves recorded from DM muscles and also a change in the post-junctional neural responses to electric field stimulation of intrinsic motor nerves. It is early to determine if there are significant and consistent changes in tissues from DM patients but with the help of this Diacomp grant to obtain RO1 funding we are continuing to collect a significant amount of data on this topic.

Kurahashi M, Nakano Y, Peri LE, Townsend JB, Ward SM, Sanders KM. A novel population of subepithelial platelet-derived growth factor receptor α-positive cells in the mouse and human colon. Am J Physiol Gastrointest Liver Physiol. 2013 May 1;304(9):G823-34.

2. Publications:

We do not have any publications to date. However we plan to submit several abstracts for next years Digestive Disease Week and we are preparing several publications for submission. The Diabetic Complications Consortium (DiaComp) Pilot & Feasibility program grant will be acknowledged in **ALL** of these and any further publications that this award supported.