

Interstitial Cells of Cajal and the Promise of Single Cell Molecular Analysis

Raúl Loera-Valencia*

Center for Alzheimer Research, Division for Neurogeriatrics Novum, Sweden

*Corresponding author: Raúl Loera-Valencia, Karolinska Institutet, Department NVS, Center for Alzheimer Research, Division for Neurogeriatrics, Novum, Blickagången 6, 141 57 Huddinge, Sweden, Tel: 46(0)8-58583751; E-mail: zazek84@gmail.com

Rec date: Apr 27, 2015; Acc date: Apr 28, 2015; Pub date: Apr 30, 2015

Copyright: © 2015 Loera-Valencia R. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Editorial

The interstitial cells of Cajal (ICC) are pacemaker cells organized in networks located in all layers of the intestines [1,2]. Together with the myenteric neurons and the smooth muscle cells, they comprise the machinery that controls the peristaltic movements [3,4]. However, the individual contribution of smooth muscle and ICC to the different motility patterns present throughout the GI tract is not completely described [5]. This task has proven difficult because of the overlapping mechanisms to produce motility in the gut, as well as the close embryonic and anatomic relationship of ICC and myocytes, which underlies the myocyte-like differentiation of ICC after several days in culture [6] impeding the generation of pure ICC cultures and the performance of “bulk” molecular analyses.

Recent attempts to purify ICC and define their transcriptomic differences with myocytes have identified genes that can be used to discern between the anatomically distinct populations of ICC and myocytes at the molecular level [7,8]; unfortunately, the required FACS enrichment of ICC to obtain a transcript library hampers the identification of ICC subtypes with physiological functions. The existence of these subtypes has been suggested previously in W/W (v) mutant mice had a subpopulation of c-Kit⁻/Ano1⁺ ICC underlying the normal motor patterns termed “ripples” in the mouse colon [9]. Moreover, ICC subpopulations are being discovered in organs such as the bladder [10], epicardium [11] and in the embryonic intestine [12]. A recent publication point out the myogenic origin of the ripples in the embryonic GI tract, where they looked for c-Kit positive cells and recorded the electrical and mechanical activity of W/W (v) mice [13]; however in the same fashion as with the Ano1⁺ ICC in the colon, would it be reasonable to think of c-Kit⁻ ICC precursors in the developing embryo which can replace the function of the c-Kit⁺ ICC ablated by the W (v) mutation? Using single cell RT-PCR in its simplest form combined with electrophysiology and immunohistochemistry we have been able to identify subpopulations of ICC based on their expression of Kv channels in the murine colon, calling for further research into this aspect of ICC biology [14].

When the GI tract suffers from pathological insults, the ICC network is disrupted and the number of ICC in the intestine diminishes. After the injury is resolved, the ICC network is restored together with normal motility in the gut [15-17]. This phenomenon raises the question of the existence of a population of cell progenitors replenishing the lost ICC. Whether this population is part of one of the layers of the ICC or comes from the trans-differentiation of fibroblasts or myocytes are questions that can be addressed applying the new molecular tools for single cell analysis.

The electrical activity of ICC originates from the interplay between pumps and channels, generating their oscillating pacemaker activity. Although most of the conductances in ICC are known, there are

“orphan” conductances that have not a particular gene associated to them. Such is the case of the maxi channel, which has proven elusive to identify as shown by knockdown studies performed in fibroblasts [18]. To address the identity of the maxi channel, its differential expression between ICC and myocytes is an invaluable tool that harbors the potential for this particular discovery, however, the maxi channel is not present in 100% of the ICC which difficult knock-down studies. Carbachol and other agonists that elevate intracellular calcium have shown to activate the maxi channel [19] and very recent developments in spatially resolved, quantitative mRNA profiling in single cells [20-22] could detect the increase of messengers in a transcriptomic scale, allowing identification of the genetic entity –or entities- that upregulates in response to pharmacologic stimulus.

In summary, the study of ICC physiology as well as the study of GI motility can tremendously benefit from the implementation and use of the single cell molecular analysis tools available today. The accumulated knowledge in the field has produced many unique experimental settings and approaches which, combined with the use of more precise molecular tools, opens possibilities for solving questions that have stood for more than 100 years.

References

1. Huizinga JD, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, et al. (1995) W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 373: 347-349.
2. Thuneberg L (1999) One hundred years of interstitial cells of Cajal. *Microsc Res Tech* 47: 223-238.
3. Barajas-López C, Berezin I, Daniel EE, Huizinga JD (1989) Pacemaker activity recorded in interstitial cells of Cajal of the gastrointestinal tract. *Am J Physiol* 257: C830-835.
4. Huizinga JD (1999) Gastrointestinal peristalsis: joint action of enteric nerves, smooth muscle, and interstitial cells of Cajal. *Microsc Res Tech* 47: 239-247.
5. Garcia-Lopez P, Garcia-Marin V, Martínez-Murillo R, Freire M (2009) Updating old ideas and recent advances regarding the Interstitial Cells of Cajal. *Brain Res Rev* 61: 154-169.
6. Huizinga JD, Lammers WJ, Mikkelsen HB, Zhu Y, Wang XY (2010) Toward a concept of stretch coupling in smooth muscle: a thesis by Lars Thuneberg on contractile activity in neonatal interstitial cells of Cajal. *Anat Rec (Hoboken)* 293: 1543-1552.
7. Ordög T, Redelman D, Miller LJ, Horvath VJ, Zhong Q, Almeida-Porada G, Zanjani ED, Horowitz B, Sanders KM (2004) Purification of interstitial cells of Cajal by fluorescence-activated cell sorting. *American journal of physiology Cell physiology* 286: C448-456.
8. Chen H, Ordög T, Chen J, Young DL, Bardsley MR, et al. (2007) Differential gene expression in functional classes of interstitial cells of Cajal in murine small intestine. *Physiol Genomics* 31: 492-509.
9. Wang XY, Chen JH, Li K, Zhu YF, Wright GW, Huizinga JD (2014) Discrepancies between c-Kit positive and Ano1 positive ICC-SMP in the W/Wv and wild-type mouse colon; relationships with motor patterns and

- calcium transients. *Neurogastroenterology and motility: the official journal of the European Gastrointestinal Motility Society* 26:1298-1310.
10. Gevaert T, Vanstreels E2, Daelemans D2, Franken J3, Van Der Aa F3, et al. (2014) Identification of different phenotypes of interstitial cells in the upper and deep lamina propria of the human bladder dome. *J Urol* 192: 1555-1563.
 11. Suciu L, Popescu LM, Regalia T, Ardelean A, Manole CG (2009) Epicardium: interstitial Cajal-like cells (ICLC) highlighted by immunofluorescence. *J Cell Mol Med* 13: 771-777.
 12. Uyttendaele L, Shepherd IT, Hubens G, Timmermans JP, Van Nassauw L (2013) Expression of neuropeptides and anoctamin 1 in the embryonic and adult zebrafish intestine, revealing neuronal subpopulations and ICC-like cells. *Cell Tissue Res* 354: 355-370.
 13. Roberts RR, Ellis M, Gwynne RM, Bergner AJ, Lewis MD, et al. (2010) The first intestinal motility patterns in fetal mice are not mediated by neurons or interstitial cells of Cajal. *J Physiol* 588: 1153-1169.
 14. Wright GW, Parsons SP, Loera-Valencia R, Wang XY, Barajas-López C, et al. (2014) Cholinergic signalling-regulated KV7.5 currents are expressed in colonic ICC-IM but not ICC-MP. *Pflugers Arch* 466: 1805-1818.
 15. Huizinga JD (1998) Neural injury, repair, and adaptation in the GI tract. IV. Pathophysiology of GI motility related to interstitial cells of Cajal. *Am J Physiol* 275: G381-386.
 16. Huizinga JD, Zarate N, Farrugia G (2009) Physiology, injury, and recovery of interstitial cells of Cajal: basic and clinical science. *Gastroenterology* 137: 1548-1556.
 17. Wang XY, Huizinga JD, Diamond J, Liu LW (2009) Loss of intramuscular and submuscular interstitial cells of Cajal and associated enteric nerves is related to decreased gastric emptying in streptozotocin-induced diabetes. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 21:1095-e1092.
 18. Sabirov RZ, Merzlyak PG (2012) Plasmalemmal VDAC controversies and maxi-anion channel puzzle. *Biochim Biophys Acta* 1818: 1570-1580.
 19. Wright GW, Parsons SP, Huizinga JD (2012) Ca²⁺ sensitivity of the maxi chloride channel in interstitial cells of Cajal. *Neurogastroenterol Motil* 24: e221-234.
 20. Valet G (2005) Cytomics, the human cytochrome project and systems biology: top-down resolution of the molecular biocomplexity of organisms by single cell analysis. *Cell Prolif* 38: 171-174.
 21. Ståhlberg A, Thomsen C, Ruff D, Åman P (2012) Quantitative PCR analysis of DNA, RNAs, and proteins in the same single cell. *Clin Chem* 58: 1682-1691.
 22. Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X (2015) RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. *Science* 348: aaa6090.