leagues have reported that TRAF-4 protein is nuclear.[3] In contrast, we observe exclusively cytosolic immunostaining, sometimes with a vesicular/organellar pattern typical of other TRAF-4 family proteins.[4,5] Thus, although unusual for TRAF-family proteins, we cannot exclude the possibility that alternative forms of the TRAF-4 protein exist that are targeted to the nucleus.

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References

1. Krajewska M, Krajewski S, Zapata JM, Van Arsdale T, Gascoyne RD, Berern K, McFadden D, Shabaik A, Hugh J, Reynolds C, Clevenger CV, Reed JC: TRAF4 expression in epithelial progenitor cells. Am J Pathol 1998, 152:1549–1561
2. Bieche I, Tomasetto C, Regnier CH, Moog C, Rio MC, Lidereau R: Two distinct amplified regions at 17q11–q21 involved in human primary breast cancer. Cancer Res 1996, 56:3886–3890
3. Tomasetto C, Regnier CH, Moog-Lutz C, Mattei MG, Chenard MP, Lidereau R, Basset P, Rio MC: Identification of four novel human genes amplified and overexpressed in breast carcinoma and located to the q11–q21.3 region of chromosome 17. Genomics 1995, 28:367–376
4. Regnier CH, Tomasetto C, Moog-Lutz C, Chenard MP, Wendling C, Basset P, Rio MC: Presence of a new conserved domain in CART1, a novel member of the tumor necrosis factor receptor-associated protein family, which is expressed in breast carcinoma. J Biol Chem 1995, 270:25715–25722
5. Kaye KM, Devergne O, Harada JN, Izumi KM, Yalamanchili R, Kieff E, Mosialos G: Tumor necrosis factor receptor associated factor 2 is a mediator of NFκB activation by latent infection membrane protein 1, the Epstein-Barr virus transforming protein. Proc Natl Acad Sci 1996, 93:11085–11090

Interstitial Cells of Cajal: Pacemaker Cells?

To the Editor-in-Chief:

Interstitial cells of Cajal have recently been identified as pacemaker cells for gastrointestinal motor activity[1] and have been implicated in motility disorders such as pseudo-obstruction.[2] Maturation of interstitial cells of Cajal is linked to interaction between Steel factor and its Kit tyrosine kinase membrane receptor.[3] This has led to the use of anti-Kit antibodies to characterize ICC in normal and pathological tissue. Evidence now suggests that CD34-positive stromal tumors, which have been shown to be Kit-positive, may differentiate into ICC-like cells.[4,5] Kindblom and coworkers[5] extend the identification of tumors by ultrastructural examinations and suggest using the term "pacemaker tumors."

The structural identification of cells as interstitial cells of Cajal has taken almost 100 years of painstaking work with the notable efforts in recent years of Thuneberg, Rumessen, and Faussone-Pellegrini.[6,7] In fact, in the human GI tract it is still difficult to recognize ICC by electron microscopy.[8] The general consensus is that ICC do not have unique characteristics or a specific ultrastructural marker but that a list of electron microscopy features together with their relationship to smooth muscle cells and nerves have to be used to make a positive identification. The list of features usually includes the presence of caveolae, smooth endoplasmic reticulum, an abundance of mitochondria and an abundance of intermediate and thin filaments, but absence of thick filaments. However, it is recognized that there are differences among species and according to location within the same musculature. In the paper by Kindblom and coworkers, the strategy used was to identify individual features in various tumors cells such as the presence of rough endoplasmic reticulum or caveolae or prominent Golgi zones. Then the suggestion is made that these cells might be ICC. However, this is extremely risky because it is the combination of all the features in a single cell, in addition to its location, that identifies an ICC. Obviously the location as well as natural contacts with other cells are lost in tumor cells; for this reason alone, identification will be difficult. It would be misleading to identify ICC in tumors or pathological specimens using only a few ICC features, because none of these features are unique to ICC.

ICC have been identified as pacemaker cells; however, it is clearly recognized that only a few subsets of ICC function in this capacity. Other subsets of ICC have an as yet unidentified function or possibly are involved in inhibitory neurotransmission.[9,10] At the moment it appears very likely that all intestinal organs have ICC that are not involved in pacemaking activity, such as the ICC associated with the deep muscular plexus in the small intestine and the ICC dispersed within the circular muscle layer of the stomach. Hence, it seems inappropriate to call tumors that might be differentiated into ICC-like cells as pacemaker tumors if the cells cannot be linked to a specific ICC subtype. In the small intestine, for example, one could call a tumor a pacemaker tumor (if the term has any validity at all) only if the tumor is shown to have developed from or into ICC from the myenteric plexus area.

Kindblom and coworkers identify GANT tumors as a subgroup of stromal tumors with prominent axon-like cytoplasmic processes, loosely organized intermediate filaments, scattered microtubules, and occasional dense-core granules that may be found in bulbous synapse-like structures. We agree with this. Kindblom and coworkers remark that tumors with GANT-like features did not differ from other stromal tumors and that "the ultrastructural features described as being diagnostic of GANT are, in fact, characteristics of gastrointestinal pacemaker cells." Of course, interstitial cells of Cajal do not have dense core granules or synapse-like structures. However, ICC are indeed often associated with neural structures. Since Kindblom and coworkers also noted that 55 out of 78 Kit-positive tumors were immunopositive for PGP9.5, a nonspecific neuronal marker, their conclusion that the close association between ICC and nerves may be reflected in the ICC tumors seems logical. The focal GANT-like features, which can be present in CD34- and Kit-positive stromal tumors, will then be one reason for the cellular heterogeneity of these tumors.[11] At present, however, it appears judicious to classify tumors as GANT when they have predominant immunopositivity to PGP9.5 and neuron-specific enolase as well as the ultrastructural

criteria described above. Since ICC appear to be the only cells positive for Kit and CD34 as well as vimentin, tumors identified by these immunohistochemical markers could be associated with ICC. Unfortunately, ultrastructural findings in these tumors are not helpful except to confirm the heterogeneity of these tumors. Caution is still warranted since vimentin is not a specific marker, CD34 can be found in perineural fibroblasts, and the Kit gene is a proto-oncogene which obviously could be expressed in other cells.

This study by Kindblom and coworkers combines tumors from different organs without apparent recognition that there are many differences between ICC in different organs. In fact, we still need more research into the identification of ICC in many locations in the human body, as well as verification of whether or not all Kit-positive cells are indeed ICC according to electron microscopy criteria. We may also have to redefine criteria for identifying ICC when more data become available on the characteristics of ICC in the various locations.[12] In light of this, it becomes clear that identification of ICC in human tumors will be difficult, particularly if the cells are undifferentiated.

The study by Kindblom and coworkers makes a positive contribution to the characterization of stromal tumors. Based on their Kit-positivity, these tumors could be classified as ICC tumors in the tradition of the naming of other tumors. Positive identification of ICC in the tumors, however, has not yet been possible.

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References

1. Thomsen L, Robinson TL, Lee JCF, Farraway L, Hughes MJG, Andrews DW, Huizinga JD: Interstitial cells of Cajal generate a rhythmic pacemaker current. Nat Med 1998, 4:848–851
2. Kenny SE, Vanderwinden JM, Rintala RJ, Connell MG, Lloyd DA, Vanderhaegen JJ, De Laet MH: Delayed maturation of the interstitial cells of Cajal: a new diagnosis for transient neonatal pseudoobstruction: report of two cases. J Pediatr Surg 1998, 33:94–98
3. Kluppel M, Huizinga JD, Malysz J, Bernstein A: Developmental origin and Kit-dependent development of the interstitial cells of Cajal in the mammalian small intestine. Developmental Dynamics 1998, 211: 60–71
4. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad TG, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y: Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998, 279:577–580
5. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM: Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998, 152:1259–1269
6. Thuneberg L: Interstitial Cells of Cajal. In Handbook of Physiology, the gastrointestinal system. Edited by GS Schultz, JD Wood, BB Rauner. Bethesda, MD, American Physiological Society, 1989, pp 349–386
7. Faussone-Pellegrini MS: Histogenesis, structure, and relationships of interstitial cells of Cajal (ICC): from morphology to functional interpretation. (review) Eur J Morphol 1992, 30:137–148
8. Rumessen JJ, Thunberg L: Interstitial cells of Cajal in human small intestine: ultrastructural identification and organization between the main smooth muscle layers. Gastroenterology 1991, 100:1417–1431
9. Huizinga JD, Thuneberg L, Vanderwinden JM, Rumessen JJ: Interstitial cells of Cajal as pharmacological targets for gastrointestinal motility disorders. Trends Pharmacol Sci 1997, 18:393–403
10. Sanders KM: A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. (review) Gastroenterology 1996, 111:492–515
11. Erlandson RA, Klimstra DS, Woodruff JM: Subclassification of gastrointestinal stromal tumors based on evaluation by electron microscopy and immunohistochemistry (see comments). Ultrastruct Pathol 1996, 20:373–393
12. Huizinga JD: Neural injury, repair, and adaption in the GI tract. IV. Pathophysiology of gastrointestinal motility related to interstitial cells of Cajal. Am J Physiol 1998, 275:(in press)

Authors' Reply:

We appreciate the interest Drs. Huizinga el al have expressed in our article,[1] especially because some of the authors are responsible for the painstaking work that has led to our present knowledge of the anatomy, morphology, and physiology of the interstitial cells of Cajal (ICC).[2–11]

We agree entirely that the identification of ICC in the normal gastrointestinal tract in humans as well as a number of other species has been problematic and that it depends largely upon a list of electron microscopic features as well as their relationship to the smooth muscle and nerve structures. The discovery that the ICC are dependent upon stem cell factor and c-kit receptor for their normal development, maturation, and function was therefore an important breakthrough,[9] because it gave us a new way of identifying the ICC by c-kit receptor immunohistochemistry. Drs. Huizinga et al state that it would be risky to suggest that gastrointestinal stromal tumors (GIST) have phenotypic characteristics of ICC based upon a single electron microscopic feature or even a list of features, because no electron microscopic findings are truly unique for the ICC. We fully agree. Our suggestion that GIST are of ICC origin or at least differentiate toward an ICC phenotype is instead based upon: (1) light microscopic appearance, which distinguishes GIST from true smooth muscle, nerve sheath, and neural and fibrous tumors of the gastrointestinal tract; (2) the location of the tumors in the muscularis propria; (3) the occurrence of ICC hyperplasia in the immediate vicinity of the myenteric plexus in some cases; (4) the electron microscopic profile, which includes a spectrum of findings that parallel that of ICC and which, more importantly, distinguishes the tumor cells of GIST from other types of differentiated cells of the gastrointestinal muscular wall; and (5) immunohistochemical characteristics, including uniform c-kit positivity and

CD34 positivity in the majority of GIST. With the exception of the ICC, no normal cells of the gastrointestinal wall express c-kit. Furthermore, there is at least a subpopulation of ICC that normally expresses CD34. We therefore believe that the current term, "uncommitted" gastrointestinal stromal tumor, is misleading and that it does not accurately reflect our current knowledge. Our views regarding GIST are supported by the findings of Drs. Hirota et al.[12] Some of the authors of the letter to the editor also seem to agree with our opinion; Drs. Sircar, Hewlett, and Riddell presented an abstract at the same meeting in which we presented our results regarding GIST. They concluded, based solely upon immunohistochemical studies, that "most GIST arise from ICCs."[13]

We do not agree with the comment that "it appears judicious to classify tumors as GANT" when they show immunoreactivity for PGP9.5 and neuron-specific enolase. The nonspecificity of neuron-specific enolase is well known. PGP9.5 is a sensitive neural marker but is far from specific, as indicated by our study and others.[1,14] Do Drs. Huizinga et al really mean that all 55 of the 78 PGP9.5-positive GIST in our study series should be classified as GANT? The concept of GANT as an entity distinguishable from GIST is not based upon any well-established clinicopathological, prognostic, light microscopic, or even clear-cut immunophenotypical differences. It is based entirely upon a single ultrastructural finding, that is, occasional nerve cell features including synapse-like structures and dense core granules.[15–17] Considering that the ultrastructural features of many published cases of GANT are less than convincing and, more importantly, the normal abundance of nerve endings in the gastrointestinal muscle wall where these tumors originate, the foundation for GANT is quite weak. In contrast to what Drs. Huizinga et al imply, we did not observe tumor cells with nerve cell features. Instead, we reported that 6 of 58 ultrastructurally studied tumors showed only a few tumor cells "closely associated with rare bulbous, synapse-like structures containing clusters of dense core granules." Because these six tumors had a light and electron microscopic appearance otherwise identical to GIST, and were also c-kit receptor- and CD34-immunoreactive, it is more likely that the synapse-like structures represented entrapped normal structures than evidence of autonomic nerve cell differentiation in a distinctly different tumor. We maintain that tumors previously classified as GANT are probably part of the spectrum of GIST with ICC features. The objection of Drs. Huizinga et al to the nomenclature we suggested, "gastrointestinal pacemaker cell tumor," is understandable. We agree that pacemaker cell function may not be the only function of ICC, even if it remains the only well-documented function. However, in an ongoing study of very small microscopic GIST, which are sometimes seen in patients with multiple tumors, we have observed that the tumors seem to be closely related to the myenteric plexus and are sometimes associated with ICC hyperplasia around the myenteric plexus. These findings suggest that GIST may originate from those cells of the ICC system that have a pacemaker cell function. The term gastrointestinal pacemaker cell tumor (GIPACT) was also chosen to reflect the only well-documented physiological function of the ICC system and to avoid the clumsy and unusable, but perhaps more accurate, term "gastrointestinal stromal tumor with phenotypic features of the interstitial cells of Cajal."

In our article, we suggested that kit gene mutations or deletions may play a role in the development and progression of GIST/GIPACT. The recent elegant study of Drs. Hirota et al, which appeared in *Science* while our paper was in press, demonstrated that kit gene mutations led to constitutive ligand-independent activation of the receptor in five GIST.[12] Moreover, transfection of the mutant c-kit complementary DNAs induced malignant transformation of cell lines, further indicating that kit gene mutations contribute to the development of GIST. Both their study and ours clearly indicate that there is indeed a human neoplasm that corresponds to the ICC system.

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References

1. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM: Gastrointestinal pacemaker cell tumor (GIPACT): Gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998, 152:1259–1269
2. Thuneberg L: Interstitial cells of Cajal: intestinal pacemaker cells? In: Advances in Anatomy. Embryology and Cell Biology. Edited by F Beck, W Hild, J van Limborgh, R Ortmann, JE Pauly, TH Schiebler. Berlin, Springer Verlag, 1982, pp 1–130
3. Barajas-Lopez C, Berezin I, Daniel EE, Huizinga JD: Pacemaker activity recorded in interstitial cells of Cajal of the gastrointestinal tract. Am J Physiol 1989, 257:C820–C835
4. Langton P, Ward SM, Carl A, Norell MA, Sanders KM: Spontaneous electrical activity of interstitial cells of Cajal isolated from canine proximal colon. Proc Natl Acad Sci 1989, 86:7280–7284
5. Rumessen JJ, Peters S, Thuneberg L: Light and electron microscopical studies of interstitial cells of Cajal and muscle cells at the submucosal border of human colon. Lab Invest 1993, 68:481–495
6. Rumessen JJ, Mikkelsen HB, Thuneberg L: Ultrastructure of interstitial cells of Cajal associated with deep muscular plexus of human small intestine. Gastroenterology 1992, 102:56–68
7. Rumessen JJ, Mikkelsen HB, Qvortrup K, Thuneberg L: Ultrastructure of interstitial cells of Cajal in circular muscle of human small intestine. Gastroenterology 1993, 104:343–350
8. Thuneberg L: Interstitial cells of Cajal. In: Handbook of Physiology: The Gastrointestinal System Sect. 6, Vol. I, Part I. Edited by JD Wood. Bethesda, MD, American Physiological Society, 1989, pp 349–386
9. Huizinga JD, Thuneberg L, Kluppel A, Malysz J, Mikkelsen HB, Bernstein A: W/kit gene required for interstitial cells of Cajal, and for intestinal pacemaker activity. Nature 1995, 373:347–349
10. Ward SM, Burns AJ, Torihashi S, Sanders DM: Mutation of the protooncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. J Physiol 1994, 480:91–97
11. Lecoin L, Gabella G, le Douarin N: Origin of the c-kit-positive interstitial cells in the avian bowel. Development 1996, 122:725–733
12. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Tunion GM, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y: Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998, 279: 577–580

13. Sircar K, Hewlett B, Riddell RH: Most gastrointestinal stromal tumors (GIST) arise from the interstitial cells of Cajal (ICC) (abstract). Mod Pathol 1998, 11:71A
14. Thompson EM, Evans DJ: The significance of PGP 9.5 in tumours: an immunohistochemical study of gastrointestinal stromal tumours. Histopathology 1990, 17:175–177
15. MacLeod CB, Tsokos M: Gastrointestinal autonomic nerve tumors. Ultrastruct Pathol 1991, 15:49–55
16. Lauwers GY, Erlandson RA, Caspers ES, Brennan MF, Woodruff JM: Gastrointestinal autonomic nerve tumors: a clinicopathological, immunohistochemical, and ultrastructural study of 12 cases. Am J Surg Pathol 1993, 17:887–897
17. Erlandson RA, Klimstra DS, Woodruff JM: Subclassification of gastrointestinal stromal tumors based on evaluation of electron microscopy and immunohistochemistry. Ultrastruct Pathol 1996, 20: 373–393