HERG K^+ Channels and β1 Integrins Interact through the Assembly of a Macromolecular Complex

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HERG K^+ channels are distinctive channels encoded by the *herg* (*human eag-related gene*) gene, which belongs to the *eag* family.[1] Structurally, the coded protein presents six transmembrane domains (named S1-S6), one of them—the S4—being a voltage sensor, and presenting cytosolic N and C termini. The N terminus, which contains a PAS domain, strongly affects the biophysical properties of the channel. The functional HERG channels are tetramers with a pore region, permeable to the K^+ ions and responsible for the current flow through the plasma membrane.

The *herg* gene family comprises three members, *erg1*, *erg2*, and *erg3*,[2] displaying a peculiar expression pattern in different tissues; *herg1*, the first to be discovered, is the best characterized of the family. It is expressed mainly in the heart, where it contributes to Ikr, one of the currents repolarizing the cardiac action potential. Mutations in the *herg1* gene are indeed related to cardiac arrhythmias known as LQT syndromes. Moreover, the *herg1* gene is expressed in the brain and in some nonexcitable tissues. In particular, we first discovered that the *herg* gene is expressed in many tumor cell lines of different histogenesis, from rabdomyosarcoma to leukemia,[3] and in primary tumors, such as endometrial cancer.[4] In these cells, HERG channels are responsible for clamping the membrane resting potential (V_{REST}) to substantially depolarized values (around −30 mV), characteristic of proliferating, immature cells. For this reason, the functional characterization of the HERG channel and its regulation would be of particular interest, contributing to a better understanding of the role the biophysical dimension plays in the cellular signaling involved in neoplastic disease.

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Consistent with this hypothesis is our demonstration that HERG channels are regulated by integrins, which are adhesion receptors deeply involved in various aspects of the cancerous phenotype, such as cellular motility and invasion.[5] In particular, HERG channel activation is dependent upon integrin-mediated cell adhesion, both in neuroblastoma SH-SY5Y and in preosteoclastic leukemic FLG 29.1 cell lines.[6,7] Upon integrin-mediated cell adhesion to laminin (LM) and fibronectin (FN), respectively, HERG channels undergo activation, evidenced by the increase in the related current (I_{HERG}); moreover, this activation drives cell differentiation, revealed by neurite outgrowth in neuroblastoma cells and by the expression of osteoclastic markers (calcitonin receptor: CtR; tartrate-resistant acid phosphatase: TRAP; vitronectin receptor: CD51/$\alpha v\beta 3$) in leukemic cells. In both these cell lines, the activation of HERG channels is apparently sustained by $\beta 1$ integrin subunit activation, through the involvement of a pertussis toxin–sensitive G_i protein. Moreover, I_{HERG} modulates the tyrosine phosphorylation of the $pp125^{FAK}$ in neuroblastoma cells, suggesting that this kinase is functionally linked to HERG channels in the voltage-dependent step of commitment to neuritogenesis.[8]

We are now deepening our study to better define the molecular interactions involved in these models. Western blot experiments performed on these cell lines, carried out using a commercially available polyclonal antibody directed against the C terminus of the channel (Alomone Labs), revealed that the HERG protein exists in two isoforms, one full length and one truncated at the N terminus. This pattern was confirmed by Western blot experiments performed with an antibody produced in our laboratory and directed against the N terminus of the protein. It is interesting to stress here that FLG 29.1 cells express the truncated isoform to a greater extent than the full-length one; this result can apparently account for the fact that these cells show a peculiar I_{HERG}, with biophysical properties typical of those displayed by a N terminus–deleted channel.[9]

To investigate whether a physical association exists between integrin receptors and HERG channels, we performed immunoprecipitation experiments. Cells were seeded on Petri dishes coated with the appropriate extracellular matrix (ECM) protein or BSA for 1 h and then processed for protein extraction; immunoprecipitation was performed using an anti-$\beta 1$ monoclonal antibody and protein A beads; and the anti HERG C terminus antibody was used for membrane decoration following Western blot.

The results indicate that either the full-length or the truncated HERG proteins coimmunoprecipitate with $\beta 1$ integrin, and that the latter appears to be strongly modulated upon cell adhesion to the ECM. This result opens interesting questions as to the possibility of a localization of HERG channels at the focal adhesion level. Preliminary results obtained from double-staining experiments performed on SY5Y cells using the anti-HERG N terminus antibody and a monoclonal anti-paxillin antibody demonstrate the colocalization of these proteins.

As for the signaling pathway that is evoked by $\beta 1$-dependent cell adhesion and that leads to HERG channel activation, we tested the possibility of a PKA involvement, since HERG channels present four putative PKA consensus sites, three in the C terminus and one in the N terminus, which seem somehow to affect the current.[10] FLG 29.1 cells were then plated on FN or BSA for different times (from 5 min to 1 h), and the activation of PKA was assessed by means of the PepTag nonradioactive

assay (Promega). Preliminary results obtained on FN-seeded cells show an increase in PKA activity, which is maximal within 5–10 min after cell adhesion and declines to basal levels after 30–60 min.

In all, these data open interesting questions as to the possibility of a complex association between membrane proteins (HERG and integrins) and cytoplasmic components, which could integrate the signaling evoked by cell adhesion to ECM with the machinery leading to cell differentiation.

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