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Does a physiological role for KCNE subunits exist in the immune system?

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The study of channel modulation by regulatory subunits has attracted considerable attention. Evidence indicates a pivotal role for accessory proteins in the channelosome. For instance, these regulatory subunits are necessary to recapitulate in vivo ion currents and to further understand the physiological role of ion channels. KCNEs are a family of regulatory subunits that interact with a wide range of channels. We have described for the first time a molecular interaction between KCNE4 and the voltage-dependent potassium channel K_V1.3. The association of KCNE4, which alters the biophysical properties, trafficking and membrane localization of K_V1.3, functions as an endogenous dominant-negative mechanism. Since both proteins are expressed in the immune system, K_V1.3/KCNE4 channels may contribute to the fine-tuning of the immune response. Therefore, our results point to KCNE4 as a novel target for immunomodulation. KCNE4 is not the only KCNE which is expressed in leukocytes. All KCNEs (KCNE1-5) are present, and some members demonstrate modulation during proliferation and cancer. In summary, regulatory KCNE subunits are expressed in the immune system. In addition, several voltage-dependent K^+ channels, which could interact with KCNEs, are also detected. Therefore, KCNE subunits may play a yet undiscovered role in the physiology of the immune system.

Key words: voltage-dependent potassium channels, KCNE4, K_V1.3, leukocytes, regulatory subunits

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KCNEs are a group of regulatory subunits composed of 5 members (KCNE1-5). KCNE peptides are small single spanning membrane proteins (<20 KDa) which modulate a large number of voltage-dependent K^+ channels.[1] The most well characterized interaction occurs with KCNQ1 (K_V7.1) channels.[2-4] K_V7.1/KCNE1 channels recapitulate the cardiac I_{ks} current.[5] Several KCNE1 mutations, which trigger severe cardiac channelopathies, demonstrate the pivotal function of KCNE1 on cardiovascular physiology.[6-8] The implication of that the remaining KCNE peptides may contribute to the modulation of I_{ks} is now under intense investigation.[9-13] Besides K_V7.1, KCNE members associate with other K^+ channels. Thus, K_V11.1 in association with KCNE2 conducts the cardiac I_{kr} current.[14]

Although many KCNE subunits share tissue expression with Shaker K^+ channels (K_V1) channels, KCNE interactions with K_V1 channels have attracted little research.[1] Early in 1992, K_V1.3 and KCNE1 were simultaneously cloned in human Jurkat T-cells.[15] Ten years later, Grunnet et al. (2003) described that KCNE4 modulates K_V1.1 and K_V1.3 channels and their heteromeric forms. However, while K_V1.1 and K_V1.3 biophysics were affected, K_V1.1 membrane surface targeting was not altered.[16] Later, Melman et al. (2004) described that KCNE1 co-immunoprecipitated with K_V1.5, but no further research was undertaken.[17] Recently, Abbot et al. (2008) demonstrated that *kcne2*$^{-/-}$ mice exhibit altered cardiac I_{kslow}, identified an interaction between K_V1.5 and KCNE2 in murine ventricles and suggested a functional role for KCNE2 in promoting K_V1.5 surface expression.[18]

In this context, our recent contribution unequivocally demonstrates molecular interactions between KCNE4 and K_V1.3,

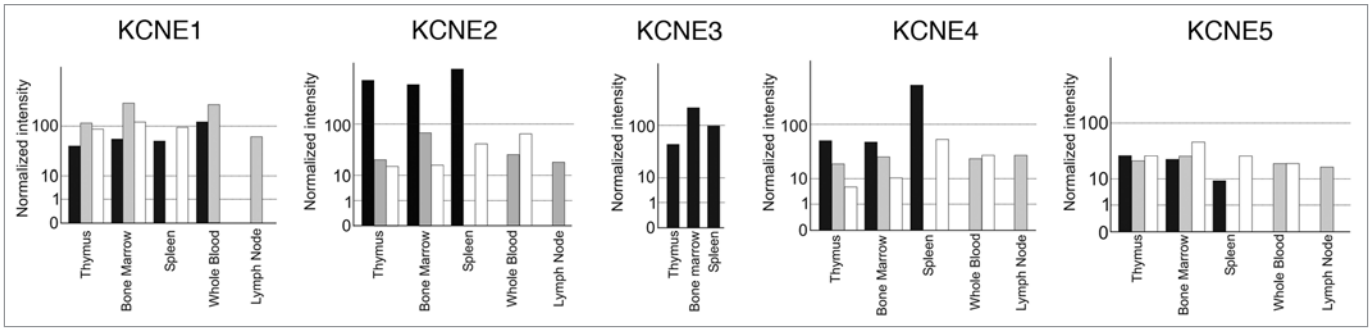

Figure 1. Human KCNE expression in healthy and cancer tissues. Affymetrix GeneChips HG-U95A-E (GeneNote, http://bioinfo2.weizmann.ac.il/cgi-bin/genenote/home_page.pl) and HG-U133A (GNF, (http://biogps.gnf.org) normalized as described in GeneCard (http://www.genecards.org). Black columns: healthy tissue (GeneNote); Grey columns: healthy tissues (GNF); White columns: cancer samples (GNF).

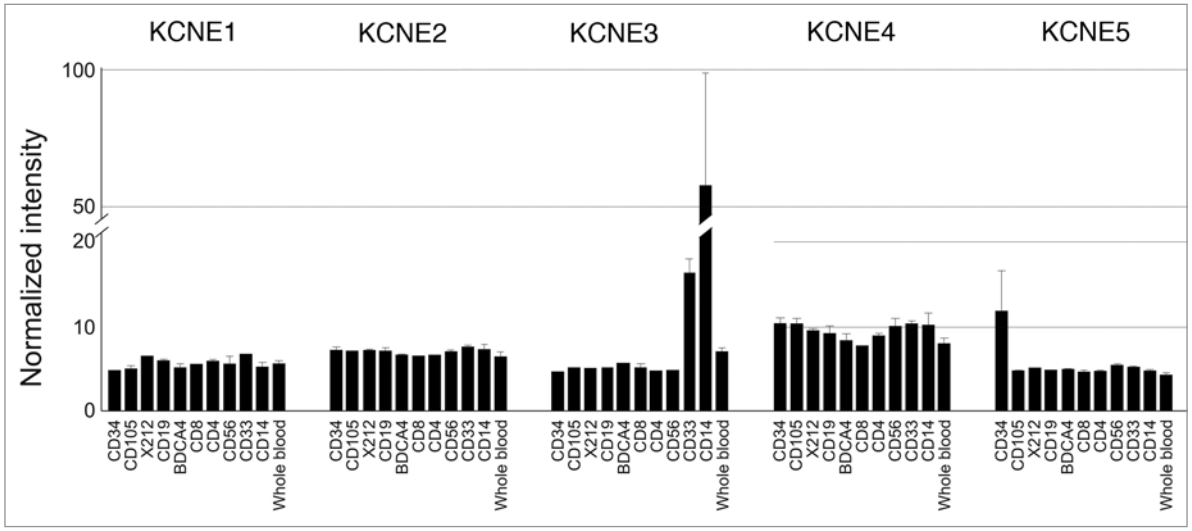

Figure 2. KCNE expression in human leukocytic cell lines. Array data from GNF BioGPS (http://biogps.gnf.org) normalized according to Su et al.[31] Legend: CD34, bone marrow CD34$^+$ progenitors; CD105, endothelial; X212, B lymphoblasts; CD19, B cells; BDCA4, dendritic cells; CD8, CD8$^+$ T-cells; CD4, CD4$^+$ T-cells; CD56, NK cells; CD33, myeloid; CD14, monocytes.

which impairs the trafficking and localization of these channels.[19] KCNE4 acts as an endogenous dominant-negative regulatory subunit. Further inhibitory mechanisms by KCNE4 have been observed. Grunnet et al. (2002) describe that KCNE4 abrogates $K_V7.1$ currents.[20] Later, George and coworkers (2008) postulate that KCNE4 could form part of a $K_V7.1$/KCNE1 heterocomplex, responsible for downregulating the I_{ks} current.[10] Furthermore, KCNE4 also inhibits the calcium-activated K^+ channel ($K_{Ca}1.1$)[21] and oligomeric $K_V4.2$ + KChIP2 channels.[22]

Although limited, the immunitary system has a defined repertoire of K^+ channel genes. Leukocytes express, $K_V1.3$, $K_V1.5$ and several $K_V\beta$ regulatory subunits.[23-25] We have demonstrated that $K_V1.3$ and $K_V1.5$ form heteromeric complexes to fine-tune the immunitary response.[23,26] The evidence that KCNE4 is also implicated,[19] the presence of KCNE1 in lymphocytes,[15] and its putative association to $K_V1.5$,[17] suggest an unidentified role for KCNE peptides in immunitary system physiology.

In fact, our recent study is not the first to describe the presence of the KCNEs in the immunitary system. KCNE1 was cloned in T-lymphocytes.[15] Grunnet et al. (2003) found KCNE4 mRNA in lymphocytes.[16] In addition, KCNEs mRNA expression has also been investigated in thymus.[10,27] Here we recapitulate the information available about the expression of KCNE1-5 in different tissue arrays, all derived from the immune system. To that end, data from tissues and tumors, cell lines and cancer cell lines are shown in **Figures 1, 2 and 3**, respectively. All KCNE1-5 members have been detected in myeloid and lymphoid lineages. It is noteworthy that while KCNE1 expression increases in some cancers, KCNE2 and KCNE4 decrease. Similarly, KCNE1 induction has also been detected in germinal tumors.[28] We have previously described the importance of $K_V1.3$ during activation and proliferation of macrophages.[25,26] Although both processes augment $K_V1.3$, the channel seems to play a dual role. Prolonged signaling during activation triggers apoptosis.[29,30] Additionally, highly proliferative cells exhibit lower KCNE4 expression

than their counterparts do (**Fig. 1**). During an insult, $K_V1.3$ is activated and sustained activation triggers cell death. The persistence of activated macrophages during inflammation leads to disease. In this scenario, KCNE4 would exert an inhibitory effect not needed in proliferation. Accordingly, it is worth noting that during proliferation, most cells become resistant to apoptosis.

Evidence demonstrates that KCNE subunits are present in the immune system and may interact with K_V1 channels. Therefore, we suggest that KCNE subunits may play a yet undiscovered role in the immune system via associations with leukocyte K^+ channels. These new interactions situate KCNE, specifically KCNE4, as novel targets for immunomodulation. Further research should be undertaken to shed some light on this new issue.

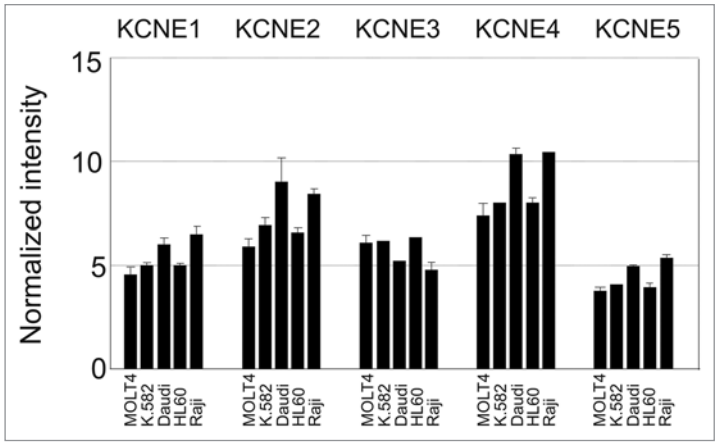

Figure 3. KCNE expression in different human leukemia and lymphoma cell lines. Array data from GNF BioGPS (http://biogps.gnf.org) normalized according to Su et al.[31] Legend: MOLT4, Lymphoblastic Leukemia; K.582, Chronic Myelogenous Leukemia; Daudi, Burkitt's Lymphoma; HL60, Promyelocytic Leukemia; Raji, Burkitt's Lymphoma.

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