Dipl.-Biol. Michal Smida

Biochemical and functional characterization of Fyn-PAG association and its role in T-cell anergy

Abstract

Anergy is an important mechanism of peripheral tolerance preventing self-reactive T cells from becoming activated and thereby the development of autoimmune disorders. Despite many attempts to identify signaling alterations responsible for the unresponsive phenotype of anergic cells, the real molecular mechanism still remains unresolved. The hallmarks of anergic cells are the upregulation of Fyn kinase and the defect in Ras activation. In human T cells, a pool of Fyn is constitutively associated with PAG and was shown to be responsible for PAG phosphorylation. Importantly, we found that PAG-associated Fyn possesses enhanced kinase activity and this leads to hyperphosphorylation of PAG in human anergic T cells. Consequently, PAG recruits more Csk and although a portion of Csk is lost upon restimulation of the cells, there is still a remarkable amount of Csk bound to PAG that leads to enhanced phosphorylation of the inhibitory tyrosine within Fyn. This mechanism might then contribute also to a block in proximal signaling, which was attributed to anergic cells.

Additionally, we have described a novel mechanism of Fyn kinase regulation. We have shown here that Fyn becomes phosphorylated not only on its C-terminal inhibitory tyrosine, but at the same time also on a tyrosine within its SH2 domain and we propose that this leads to its opened hyper-active conformation in human anergic T cells.

Furthermore, we have identified a novel multiprotein complex consisting of PAG, Fyn, Sam68 and p120RasGAP and have demonstrated an important inhibitory role of PAG on Ras activation. We have also shown that its ability to regulate Ras is dependent on both Csk and p120RasGAP association and only the deletion of both binding sites completely ablates PAGs impact on Ras signaling. Using RNA interference, we could demonstrate that suppression of PAG expression leads to an unbalanced upregulation of both Src kinase and Ras activity resulting from the loss of a critical negative feedback loop.

Finally, we have identified a new protein, which we called IGAP, as it appears to be a GTPase-activating protein that is induced only upon activation of T cells. We hypothesize that IGAP may be needed by the activated T cells to shut down their activation status in order to terminate the immune response. We could show that, contrary to p120RasGAP, IGAP is

already prelocalized at the plasma membrane and that one mechanism of its regulation might be mediated via its association to PAG.

In conclusion, we have established PAG as a potent negative regulator of Ras activation recruiting two RasGAPs and we propose that this regulatory mechanism may play a role in anergy. Thus, PAG is involved in the regulation of both Src kinases and Ras, two important oncoproteins implicated in many forms of cancer. Therefore it is tempting to speculate that there might be alterations in PAG expression and/or various PAG mutations found in certain types of cancer that might be responsible for dysregulation of cellular signaling leading to a pathological transformation of the cell.