A role for PKCδ in foam cell formation?

With great interest we have read the recently published article by Lin et al.1 where PKCδ is described to regulate uptake of oxLDL and the resultant foam cell formation, thereby suggesting a potential role of this protein kinase in atherogenesis. In their study Lin et al. used two cell types, THP-1 monocytic cell line and primary human macrophages, as models for oxLDL uptake and in vitro foam cell formation. Employing RNA interference they decreased expression of PKCδ by an estimated 40–75% in both cell types to demonstrate the effect of the kinase on lipoprotein uptake and accumulation. In THP-1 cells as well as human macrophages they observed lower oxLDL uptake. Silencing of PKCδ led to decreased expression of Akt and phosphorylation of ERK, which according to proposed model regulate together with PI3K the expression of scavenger receptors SR-A and CD36 that had previously been implicated in oxLDL uptake. Additionally, they applied rottlerin as a PKC δ inhibitor to further support their findings based on knock-down experiments.

However, the interpretation of the results is complicated by a prominent parallel decrease in protein levels of another kinase, PKCβ, which they also observed. Although Lin et al. argue that this cannot simply be due to off-target effects of the used shRNA on PKCβ because PKCβ mRNA levels are not affected, they do not exclude a role for translational repression of PKCβ, which has been described as an alternative mechanism of siRNA-mediated knockdown.²,³

In any case, the question arises whether PKCβ can actually be held responsible for regulating oxLDL uptake and foam cell formation. Or is it rather the effect of silenced PKCβ protein expression, which by itself has been known to regulate uptake of modified lipoproteins?² Actually, our own unpublished data obtained by silencing PKCβ expression in monocytic cell line and primary human macrophages (50–75% decrease in protein levels) show that knockdown of PKCβ does not cause any detectable reduction in oxLDL uptake. Moreover, we did not reveal any effect of PKCβ knockdown on PKCβ protein expression. However, rottlerin was still able to lower the uptake of oxLDL to ~50% when compared with non-treated control cells. The latter can be explained by the various PKCβ-independent effects of rottlerin, which have been well documented.⁵

Therefore, we feel that the role of PKCδ in the process of oxLDL uptake and foam cell formation is still questionable and that further investigation would be required to clarify this.

References

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A role for PKCδ in foam cell formation: reply

We would like to thank Drs Katka Szilagyi and Timo K. van den Berg for their interest and comments regarding our recent report.¹ We also greatly appreciate sharing with their interesting and unpublished results about PKCδ.

Indeed, the approach with siRNA can result in changes in protein levels of certain untargeted genes that are unrelated to silencing of the targeted gene.² However, in our study, we used different sequences of shRNAs or siRNAs against PKCδ which shared no cross-recognition with PKCβ in different cell models (THP-1 cells and human primary macrophages) and showed that knockdown of PKCδ reduced PKCβ protein expression. Interestingly, it has been shown that there is compensatory increases in PKCβ expression and activation in hearts of PKCδ knock-out mice.³ It is proposed that the cross-talk among PKC isoforms may be key for the functional integration of the signalling networks.⁴,⁵ Altogether, these results indicate that off-target effects are not necessary to be the only explanation for the observed phenomenon.

We have to emphasize that ‘Employing RNA interference they decreased expression of PKCβ by an estimated 40–75% in both cell types...’ is not completely accurate. Our study shows that the knock-down of PKCβ (~50%) in human primary macrophages results in a 15% decrease in OxLDL uptake (Figure 4A and F).¹ However, when the expression of PKCδ is reduced >90% by the aid of the lentiviral-vector transducing shRNA against PKCδ, we observe a more profound reduction in OxLDL uptake in THP-1 macrophages (50–60%; Figure 1A and S1).¹ The results suggest that the remaining level of PKCδ after the knock-down procedure is critically related to the inhibitory potency on OxLDL uptake in macrophages. This may explain in part why Drs Katka Szilagyi and Timo K. van den Berg did not observe a decrease in OxLDL uptake in their models.

Although rottlerin has been widely used as a PKCδ inhibitor in many excellent studies,¹,³–⁷ we do agree that rottlerin is not an ideal inhibitor of PKCδ because of its potential PKCδ-independent effects.⁶ Unfortunately, to the best of our knowledge, we could not find any better chemical inhibitors for PKCδ. Given this limitation, our study results remain supporting the roles of PKCδ in regulating foam cell formation that is definitely different from that of PKCβ. It has been demonstrated that PI3K/Akt and ERK signalling pathways are crucial in the uptake of modified lipoproteins and foam cell formation in human macrophages.⁷,⁸ PKCδ regulates the expression of SR-A and CD36 through PI3K/Akt and ERK signalling pathways; however, PKCβ downstream of ERK and PKCδ, regulates the expression of SR-A, but not CD36. In addition to the results in using siRNA and shRNA approaches, the down-regulation of both SR-A and CD36 is also confirmed in THP-1 macrophages expressing dominant negative PKCδ construct (Figure 1F). We thus do not agree with the suggestion that the observed reduction in OxLDL uptake in our study is simply due to the decrease in PKCβ although it remains
possible that the reduction in PKCδ may exert certain effects on the overall detected events. Collectively, we acknowledge that further in vivo investigations are needed to elucidate the real roles of PKCδ on foam cell formation. The approaches such as isolation and evaluation of macrophages from PKCδ null mice, as well as transplantation of bone marrow from PKCδ null mice to LDL receptor knockout mice may help to evaluate the impact of PKCδ on atherosclerosis. Together with growing evidence suggesting that PKCδ is also involved in lipid metabolism, we are confident that our study provides critical evidence supporting the important roles of PKCδ in foam cell formation.

References