4.1 Summary

The role and function of phospholipase D in cellular proliferation, differentiation and apoptosis is still not understood. Here, I describe the regulation of phospholipase D in some aspects of these cellular processes and the possible therapeutic intervention in one of these pathways.

The role of tyrosine phosphorylation in regulation of phospholipase D1 was investigated using a protein tyrosine phosphatase inhibitor. The induced accumulation of tyrosine phosphorylated proteins was accompanied by increased phospholipase D1 activity *in vitro*. Immunoprecipitation demonstrated that phospholipase D1 is not directly tyrosine phosphorylated in HaCaT keratinocytes in contrast to HL60 cells. These effects can be abolished using a protein tyrosine kinase inhibitor. As shown by Ras overexpressing HaCaT keratinocytes, the Ras/Raf/mitogen activated protein kinase pathway is not involved. This shows that tyrosine phosphorylation is a pathway indirectly regulating phospholipase D1.

Transcriptional regulation of phospholipase D in ceramide-induced apoptosis affects phospholipase D1. I could show that several differentiation-associated genes including phospholipase D1 are downregulated whereas members of the AP-1 transcription factor are upregulated. AP-1 is involved in the expression of differentiation-associated genes but the data presented suggest that the subunits which are upregulated in apoptosis form a repressor. In contrast, levels of phospholipase D2 mRNA are unchanged.

Moreover, I demonstrate that malignant melanoma exhibits augmented phospholipase D1 activity in comparison to primary cultured melanocytes. This increase is attained through enhanced protein expression in degenerated cells. In addition, although protein kinase Cα expression is not altered, activation of phospholipase D1 by phorbol ester is marginal in melanocytes in contrast to melanoma cells suggesting loss of an inhibitory factor of this interaction.

Cytoskeletal reorganisation is important for metastasis, and phosphatidic acid and the phospholipase D1 regulating Rho family proteins are involved in this process. Pamidronate interferes with Rho action via altering its membrane association. Here, I can show that pamidronate strongly induces apoptosis in melanoma cells. Addition of farnesol or geranylgeraniol reduces apoptosis by pamidronate with geranylgeraniol being a stronger inhibitor. This suggests geranylgeranylation being the more important step in pamidronate-induced apoptosis. However, one of the cell lines tested, Mel2A, is resistant against pamidronate

mediated inhibition of proliferation and induction of apoptosis. This resistance is not achieved by alteration of the bax/bcl-2 ratio as was shown by bcl-2 overexpressing A375 cells. The levels of RhoB mRNA expression show no significant difference between melanoma cells and melanocytes. RhoA and RhoC in contrast are elevated in melanoma cells underscoring them as possible therapeutical targets. Although cytosolic RhoA levels are increased in all four melanoma cell lines after pamidronate treatment, levels of membrane-bound RhoA are not or only slightly altered. This suggests that decreased geranylgeranylation is counteracted by increased expression of Rho proteins. It remains to be investigated if the metastatic potential of these cells is nevertheless decreased.