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A MISSING LINK BETWEEN LIPID METABOLISM, INFLAMMATION AND APOPTOSIS: PHOSPHOLIPASE A_2-ACTIVATING PROTEIN (PLAA)

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by

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Dedication

I dedicate this dissertation to Zhen Hou, M.D., my grandmother,

who has supported and inspired me throughout these years.

My dear Grandma, I know you are still there watching over me.

Without you, I would have never made it here.

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Phospholipase A_2-Activating Protein (PLAA) is a novel signaling molecule that regulates the production of arachidonic acid (AA), prostaglandin E_2 (PGE_2) and TNF-α. Literature suggests that PLAA could be involved in inflammatory responses and apoptosis. However, the *in situ* function of PLAA is elusive. To elucidate PLAA's role in TNF-α-induced inflammatory responses and cisplatin-induced apoptosis, we manipulated the expression of the *plaa* gene at cellular level using overexpression and siRNA approaches. We generated HeLa (Tet-off) cells overexpressing *plaa* (*plaa* ^{high}) and control (*plaa* ^{low}) cells. We compared *plaa* ^{high} and *plaa* ^{low} cells for transcriptional profiling and their responses to TNF-α stimulation. Overexpression of the *plaa* gene induced the expression of

the proinflammatory cytokine IL-32 and reduced the expression of annexin A4 (a PLA_2 inhibitor) and clusterin. We demonstrated that extracellular clusterin limited the production of PGE_2. We showed that upon TNF-α stimulation, *plaa* ^{high} cells revealed enhanced PLA_2 activation, COX-2 expression and PGE_2 production. Furthermore, we found that in response to TNF-α, *plaa* ^{high} cells had significantly enhanced activation of NF-κB and production of IL-6, compared to the TNF-α-stimulated *plaa* ^{low} cells. To understand regulation of *plaa* gene expression, we used a luciferase reporter system in normal HeLa cells and identified one stimulatory element, with Sp1 transcription factor-binding site, and one inhibitory element, in exon 1 of the *plaa* gene. To determine the role of PLAA in apoptosis, we compared the apoptotic responses to cisplatin in *plaa* ^{high} and *plaa* ^{low} cells. Cisplatin-stimulated *plaa* ^{high} cells contained significantly higher levels of DNA fragmentation, caspase activities, PLA_2 enzyme activity and mitochondrial damage than did the cisplatin-stimulated *plaa* ^{low} cells. siRNA against PLAA (siRNA-PLAA) reverted the above mentioned trend and promoted cell viability. Further, cisplatin-stimulated *plaa* ^{high} cells produced less cytoprotecive clusterin and more pro-apoptotic IL-32 than did the cisplatin-stimulated *plaa* ^{low} cells. siRNA-PLAA promoted clusterin production and inhibited IL-32 expression from both *plaa* ^{high} and *plaa* ^{low} cells. Finally, our proteomic analysis revealed that cisplatin-stimulated *plaa* ^{high} cells contained higher levels of phosphorylated JNK/c-Jun and FasL than did cisplatin-stimulated *plaa* ^{low} cells.