CHAPTER 4

DISCUSSION AND CONCLUSION

4. 1. Discussion

Using CM5 anti-mouse p53 polyclonal antibody to performed Western blot analysis of human lung tumor tissues, we fortuitously discovered that this antibody recognized a human protein with an apparent molecular weight of 95 kDa, which was frequently overexpressed in tumor tissues. This 95 kDa human protein was immunoprecipitated using CM5pAb, resolved through SDS-PAGE, excised from gel and subsequently subjected to protein identification using LC-MS/MS. The mass spectrum data suggested two possible candidate proteins, which were GRP-78 and glucosidase II. The western blot analysis showed that glucosidase II migrated at the same position as the CM5-reactive protein. Therefore, immunoprecipitation was performed using anti-glucosidase II mAb, subsequently the immunoprecipitated glucosidase II was resolved through SDS-PAGE, transferred onto PVDF membrane and detected with CM5pAb. The result showed that the immunoprecipitated glucosidase II was recognized by the CM5pAb thus confirming that glucosidase II and the CM5pAb reactive protein was in fact the same protein.

Glucosidase II plays a key role in the processing of N-linked oligosaccharide chains of glycoprotein and is involved in the quality control mechanism of glycoprotein folding [67]. However, to our knowledge overexpression of glucosidase II in tumor tissues had not been previously described. Of 37 lung tumor samples examined, 35 samples were found to possess an increase level of glucosidase II in

comparison to normal corresponding tissues. The high frequency of glucosidase II overexpression despite tumor stage indicates its crucial role in early stage of tumor development, at least in lung. ER is the site for folding of protein destined for different compartments of the cell. As newly synthesized proteins enter the ER lumen, they are covalently modified by the addition of a pre-assembled oligosacharide composed of 2 N-acetylglucosamines, 9 mannoses and 3 glucoses. Glycosylation of the newly synthesized protein is believed to help increasing hydrophilicity of the un-structured proteins. Subsequently, the glycosylated protein chains enter a glycoprotein-dedicated chaperone system comprising calnexin and calreticulin [(68)]. Access to calnexin/calreticurin system requires removal of the two outer most glucose residues by the sequential action of glucosidase I and glucosidase II, respectively [(69)]. Removal of the third glucose is also mediated through glucosidase II action, which is required in order to dissociate folding substrates from calnexin and release of the native proteins from the ER and transport to their final destination. However, if the protein is improperly folded, the folding sensor UDP-glucose:glycoprotein glucosyltransferase-1 (UGT1) will add back a terminal glucose to promote re-association of non-native protein and calnexin, thus prolonging their time in the ER folding environment. Cycles of de-/re-glycosylation might be extended until the protein released from calnexin fulfills the quality requirement (reviewed in (67)). Therefore, balance of glucosidase II and UGT1 activity is crucial in order to maintain protein quality control of the ER.

Disturbance in the folding capacity of the ER caused by a variety of endogenous and exogenous insults could initiate a cellular stress condition known as ER stress. ER stress is initially induced to re-establish ER homeostasis through the

activation of an integrated intracellular signal transduction termed as unfolded protein response (UPR). However, when ER stress is too severe or prolonged, the prosurvival function of the UPR turns into toxic signal, which is predominantly executed by mitochondrial apoptosis [70]. Since glucosidase II plays a major role in releasing proteins from the ER folding system, cells with overexpression of glucosidase II may impervious to ER stress. Even overloaded with misfolded proteins, the UPR and apoptosis may not be triggered at all in glucosidase II overexpressed cells. In agreement with this hypothesis, inhibition of glucosidase II has been reported to reduce proliferation and induce apoptosis [71, 72] of tumor cells. There is increasing evidence that the UPR was compromised in a large variety of human tumors (reviewed in (73)). More importantly, several recent studies have demonstrated that interfering with the activation of different arms of the UPR (i.e., PERK-eIF2α-ATF4 axis) or altering the levels of the ER molecular chaperone GRP78/BIP (a master regulator of ER function and the UPR) can inhibit tumor growth [74-76]. This evidence indicates that not only the UPR was compromised in tumors but that it contributed to survival and growth of the cancer cells. Therefore, inhibitors of glucosidase II could also be regarded as a potential anticancer agent [77, 78].

The fact that the induction of glucosidase II protein level in tumor tissues was discovered through the use of antibody against p53 indicates their structure similarity, and prompts us to try to describe the connection between these two proteins. We have demonstrated that the pattern of changes of their protein levels in response to ER stress and genotoxic stress were similar between p53 and glucosidase II. Protein levels of both p53 and glucosidase II was increased in response to UV irradiation, but decreased in response to tunicamycin-induced ER stress. However, while p53 was

translocated into the nuclease in response to UV irradiation, glucosidase II remained within the cytoplasm. Nevertheless, levels of these two proteins were decreased and become undetectable by western blot in response to ER stress, thus the sub-cellular localization of p53 and glucosidase II under this type of stress was unclear. Interestingly, there were smaller protein bands recognized by the anti-glucosidase II mAb whose nuclear levels were increased in response to both types of stress. Although we cannot rule out the possibility that these proteins may be the degradation products of glucosidase II, the induction of these bands despite levels of the full-length protein suggesting that they might be involved in the process of stress response. Nevertheless, further investigations with better technology, i.e. immune-fluorescence are needed in order to verify and confirm our observation.

The p53 tumor suppressor protein becomes stabilized, activated and trans-localized into the nuclease in response to a number of stressful stimuli, i.e., hypoxia, nucleotide depletion or oncogene activation [(79)]. The main forms of stress that activate p53 is genotoxic stress [(80)], which if left unchecked can lead to loss of genomic integrity and cancer development. Activation and nuclear translocation of p53 allows it to carry out its function as a tumor suppressor protein through a number of controlling endpoints i.e., cell cycle arrest or apoptosis [(81)]. However, it was not recognized until recently that the ER stress also plays a pivotal role in modulating p53 activity. Protein level[(82)] and function[(83)] of p53 has been reported to be reduced or suppressed in response to stress of the ER. This inhibition is believed to help ensure that p53 activation is restricted to agents that induce genotoxic stress [(84)].

However, if the ER stress is not properly responded or the UPR being compromised by altering level of regulator proteins, i.e. glucosidase II, this might lead

to the overproduction of the misfolded proteins which may be oncogenic and together with the suppression of the p53 function; the cell may be prompted to finally become cancergenic. The fact that both p53 and glucosidase II respond to ER stress and genotoxic in a similar fashion indicates the possible connection between the two proteins, which awaits further investigation.

4. 2. Conclusion

This thesis has successfully demonstrated that the human unknown protein reacted with the CM5pAb, a rabbit polyclonal antibody raised against p53 of mouse origin, is in fact an ER-resident protein glucosidase II**.** Glucosidase II was found to over-express in almost all of lung tumor tissues examined with only minimal expression normal adjacent tissues, which to our knowledge, has not been previously described. In addition, expression level of glucosidase II was altered in response to stress signals suggesting that the induction of this protein, observed in tumor tissues, may crucially play a role in driven cellular transformation rather than being just a consequent of it. Protein level of glucosidase II was frequently increased in lung tumor tissues, thus representing a valuable biomarker in aiding screening and/or diagnosis of lung cancer. For example a highly sensitive glucosidase enzymatic assay system could be developed to measure glucosidase activity from non-invasive samples i.e., exhaled breath condensate (EBC) or pleural effusion in order to screen for lung cancer. However, further investigations on the underlying mechanism of the protein induction and its relationship with p53, genotoxic stress, ER stress and tumor development are warranted.