

CHAPTER IV

DISCUSSION

Carcinogenesis is the process by which normal cells are transformed into cancer cells. This process is caused by the mutation of the genetic material which governs proliferation and cell death in normal cells. In order to get further elements of an approach to understanding cancer based on cell-level selection and evolution, the gastric cancer cell line (RGK1) was selected by continuously expose the rat normal gastric mucosal cell (RGM1) to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The analysis of specific growth rate of the RGK1 and their biochemical changes were studied in comparison with its corresponding normal cells. Contrary to the literatures, this study clearly show that RGK1 cell has specific growth rate (γ) equal to $0.0417 \pm 0.0006 \text{ h}^{-1}$ slightly lower than that of RGM1 ($\gamma = 0.0495 \pm 0.0017 \text{ h}^{-1}$). However, the RGK1 cell produced higher levels of ROS_i than its parental RGM1 cells. This is consistency to the previous reports that cancer cells produce higher levels of ROS than normal cells due to increase metabolic stress and proliferative capacity (Szatrowski and Nathan, 1991, Schumacker, 2006, and Trachootham *et.al.*, 2006). We hypothesize that the slower growth rate of RGK1 cells might be originated from the alteration of the mitochondrial antioxidant system particularly Mn-SOD. Either lacking of Mn-SOD contents or activity should be directly responsible to an increase in the superoxide anion free radical, the origin of ROS_i accumulation in mitochondria. These might cause an impaired mitochondrial function of the cancer cells. Since huge

reports mentioned that mutations in nuclear or mitochondrial genes encoding components of the mitochondrial electron transport chain (ETC) can lead to an increase in ROS generation (Wallace 2005 and Indo *et al.*, 2007). It has been demonstrated both *in vivo* and *in vitro* that antioxidant enzyme levels are altered in cancer cells. The most consistent finding in biochemical studies has been that Mn-SOD, a mitochondrial antioxidant enzyme, is lowered in most types of primary cancers and cancer cell lines examined (Oberley and Oberley, 1997). The antioxidant enzymes in human lung (Coursin *et al.*, 1996), renal (Oberley *et al.*, 1994 and Oberley *et al.*, 1996) and prostate cancer (Bostwick *et al.*, 2000 and Oberley *et al.*, 2000); immunoperoxidase studies demonstrated low levels of anti-oxidant enzyme in primary tumor, although small groups of cancer cells, often on the invading edge of the tumor, did occasionally show strong positive. The reduced level of Mn-SOD activity in cancer cells is partly due to a defect in the promoter region of the gene (Xu *et al.*, 1999). However, primary cancer from some organs has been shown to have a high level of Mn-SOD. There are studies providing evidence that Mn-SOD acts as an oncogene. Several groups showed that Mn-SOD protein is overexpressed in oral (Lo *et al.*, 2007), breast (Tsanou *et al.*, 2004), gastric and colorectal cancer tissue (Nozoe *et al.*, 2003, Kim *et al.*, 2002 and Malafa *et al.*, 2000).

Our results show that both RGM1 and RGK1 has very low Mn-SOD activity cannot measure by the technique used in this study while their stably transfected with the pCR3.1-Uni plasmids containing a sense human Mn-SOD cDNA insert were highly express high levels of Mn-SOD. It is of important to note that the additional Mn-SOD was designed to send into the matrix of mitochondria. An increase in Mn-SOD caused an increase in γ in all transfected RGK1 clones but decreased in γ all

transfected RGM1 clones. These should be interpreted as an increase in matrix Mn-SOD of mitochondria of the cancer cells should decrease in the superoxide anion free radical, thus render the mitochondria to a polarized form and viability of the cells. For normal cells, under the Mn-SOD transfected conditions may undergo growth arrest. Contrary Epperly reported that an increase in Mn-SOD protects normal tissue against oxidative stress (Epperly, 2002). In fact an imbalance of antioxidant enzymes and overexpression of Mn-SOD reportedly inhibits the proliferation of a wide variety of cancer types (Oberly, 2001, Policastro *et al.*, 2004, Chen 2005, Oberly, 2005), strong pointing to Mn-SOD as a tumor suppression protein. The high level of Mn-SOD have been associated with poor survival in multiple malignancies including glioblastoma (Ria *et al.*, 2001), gastric cancer (Kim *et al.*, 2007) and colorectal carcinoma (Nozoe *et al* 2003). The similar reports for prostate carcinoma cells (DU145) overexpressing Mn-SOD have cell growth rate that correlate with ROS level (Li *et al.*, 1999). The reduced level of Mn-SOD activity in human cancer cells is not due to a defect in primary structure of the Mn-SOD protein, a change in the dosage of the Mn-SOD gene, or a decrease in the stability of Mn-SOD mRNA in cancer cells, but rather is due to defects in the expression of the gene (St. Clair and Holland, 1991).

The amount of intracellular reactive oxygen species (ROS_i) was determined using a fluorescence probe, HPF, which allows the selective detection of hydroxyl radicals under a confocal microscope. The HPF staining of the cells can not only reflect the ROS_i levels but also provide the evidence of vacuole formation in the cytoplasm. Increased Mn-SOD did not affect the ROS_i content in transfected RGM1 cells while significantly done in transfected RGK1 cells. The ROS_i levels of transfected RGM1 did not significantly change while dramatically decreased of

RGK1 cells in the presence of quercetin. It should be noted that in a dose dependent manner of quercetin, some vacuoles excluding HPF appeared in the cytosol of RGK1 cells. In all cells studied have ROSi level that correlates with the 4-HNE protein adducts. Since the 4-HNE protein adducts were observed at perinuclear of cells probably in the acidic organelles including lysosomes and endoplasmic reticulum. The HPF staining of living cells can also provide the evidence of vacuole formation in the cytoplasm. The micrographs revealed that the 4-HNE protein adducts probably found inside the vacuoles. These results suggested that in the chemical stress originated the cellular lipid peroxidation. The 4-HNE a highly toxic aldehyde, a product of lipid peroxidation that can undergo protein adduction, entrapped and digested by acidic organelles such as lysosomes and endoplasmic reticulum following exocytosis of cells. When an abundant of 4-HNE protein adducts was found, lysosomes should fuse with autophagosomes to form autolysosomes the characteristic of autophagy. The results also clearly show that at high density and size of autophagosomes will cause an arrest of cell growth thus undergo cell death later. The autophagic induction of cells very close phenomenon and cannot distinguish from the late apoptosis/necrotic cell (type (III)). The autophagy was almost found in the Mn-SOD transfected RGK1 cells particularly in the presence of quercetin. The results suggested that the expression of Mn-SOD can play important role in regulating the autophagy of cancer cells.