Role of Oxidative Stress and Autophagy in Progression of Urothelial Carcinoma of Bladder

Introduction and Objectives: Autophagy is a conserved process that may serve to regulate turnover of aberrant organelle to maintain the normal cellular homeostasis. Recent studies indicate that tumor suppressor function of autophagy is mediated by removal of damaged oxidative organelles. Conversely, autophagy in some cancer can promote cell survival. In oxidative stress, ROS are proposed to play a crucial role in tumor progression and may modulate tumor microenvironment in favor of cancerous cells. Recently, ROS has been implicated in autophagy biology. Since the role of autophagy in urothelial carcinoma is not well understood therefore we wanted to study the status of autophagy in different grades of bladder cancer and correlated the autophagy with oxidative stress induced by mitochondria electron transport chain (mETC) inhibitors in urothelial carcinoma.
Materials and Methods: This study was conducted on normal tissues (n=15) and bladder cancer tissue (n=30) taken from bladder cancer patients and T-24 bladder cancer cell. Primary tissues (n=3) were decellularize and thereafter cultured to expand. Autophagic vesicles were observed in tissue samples (n=3) by electron microscopy (EM). This observation was correlated with the expression of autophagic markers, LC3II and Beclin by Western blot. In addition, mETC inhibitors (Rotenone, TTFA and antimycin A) were used for stimulation of oxidative stress and autophagy induction. Mitochondrial alteration was measured by DCF-HDA, Nonyl acridine orange. Cell viability was measured by annexin/7-AAD and propidium iodide RNase.
Results: The EM examination of tumor sections of high grade from patient of histopathologically proven cases of bladder cancer showed increased mean number of autophagic vesicles when compared to normal bladder tissue ($p<0.01$). Western blot also confirmed the increased expression of autophagic proteins ($p<0.03$). Further, we found that LC3II expression in primary cultured cell and T-24 was significantly increased by mETC inhibitors ($p<0.001$). mETC inhibitors increased the production of ROS ($p<0.05$), mitochondrial mass ($p<0.02$) and autophagy ($p<0.001$) whereas, N-acetyl L-cystein treatment inhibits autophagy ($p<0.0001$) and leads to apoptotic cell death ($p<0.01$).
Conclusions: Our results showed that oxidative stress in urothelial carcinoma induces autophagy through ROS mediated pathway. Therefore, autophagy appears to be associated with cell survival and tumor progression in urothelial carcinoma.