Abstract

Thesis title: Study of Gene Expression in Long Term Exposure to Hypoxia in Human Cancer Cell Lines

***Introduction*:** Glioblastomas (GBMs) are aggressive tumor of brain, generally considered to occur from glial cells. A division of GBMs develops from lower-grade gliomas and can thus be classified as "secondary," whereas some of these arise with no prior evidence of a lower grade tumor and can be classified as "primary" (Tso CL et al, 2006). Low oxygen or hypoxia is readily recognizable of rapidly proliferative GBMs and other solid tumors. Hypoxic microenvironment is of major clinical importance as it promotes both tumor progression and resistance to therapy (Vaupel P & Mayer A, 2007). From a biological and clinical point of view, the secondary GBMs are less aggressive and slow growing hence more expose to hypoxic micro-environment for a longer time whereas primary GBMs are aggressive and fast growing. Several studies including Tso CL et al, (2006) have defined gene expression differences that distinguish primary and secondary GBMs.

Tumors are likely to be highly heterogenous with both acute/intermittent hypoxia (such as during blood vessel occlusion and reperfusion events) and chronic/prolonged hypoxia (such as tumor regions distant from blood vessels (Coleman CN, 1988). Oxygenation in solid tumor varies from 8% to 0.1% or near anoxia (Brown JM, 2007; Vaupel P et al, 2007). Most *in-vitro* studies on gliomas cell lines are reported on exposure to short term hypoxia. To the best of our knowledge, there are no reports on cells exposed to long term hypoxia.

Hence, the establishment of an *in-vitro* model of long term hypoxia and comparison of short and long term exposure would:

- provide a better representation of *in vitro* conditions
- help in identifying the differences between short and long term hypoxia
- hence establish the validity of extrapolating the results of short term hypoxia *in-vitro* conditions

Materials and methods: For this study, we selected two GBM cell lines (U87MG and U373MG) and cultured under both normoxia (20% oxygen) and severe hypoxia (0.1% oxygen) for short (24 hr, 48 hr and 72 hr) and long (1 week, 3 weeks and 6 weeks) term.

Results: In our study, we observed that U87MG cells showed activation of different adaptive pathways in short and long term hypoxia. Glycolysis pathway genes were more up-regulated in short term hypoxia as compare to long term hypoxia. Different apoptotic pathways were observed to be involved in short and long term hypoxia. Extrinsic pathways genes showed down-regulation under hypoxia which was more in long term hypoxia. Intrinsic pathways genes were involved in apoptotic mechanism causing cell death. Similar to apoptosis and glycolysis, angiogenic genes also showed different expression under short and long term hypoxia.

On the other hand, different behavior of HIF-1α was observed in between two GBM cell lines varying in their p53 status. The transcript level of HIF-1α in hypoxic U87MG was lower than its normoxia while, the U373MG showed a higher transcript level under similar conditions. The transcript levels of hypoxia adaptive and HIF-1 regulated genes related to glucose metabolism, angiogenesis and cell survival were observed to have increased transcripts in both U87MG and U373MG cell lines. At protein level, U87MG showed stabilization of HIF-1α uptil 24 hrs under severe hypoxia while it was observed uptil 6 wks in U373MG. To study the role of HIF-1 in the expression of its downstream or hypoxia adaptive genes, we knocked down its function by using siRNA against HIF-1α. No significant changes in HIF-1 downstream genes expression were observed in U87MG cells, while U373MG showed the more than 75% reduction in downstream genes expression. These results indicate the role of a transcription factor other than HIF-1 in regulation of hypoxia adaptive genes under severe hypoxia. Later, we observed an increased transcript level and stabilization of HIF-2α under severe hypoxic conditions.

Furthermore, we also observed the involvement of p53 in regulation of HIF-1α under severe (0.1% oxygen) hypoxia but not in mild (5% oxygen) and moderate (2% oxygen) hypoxia.

Conclusion: Thereafter, we conclude that under hypoxic conditions, cells adapt differently depends on several factors including duration and degree of hypoxia, cell type and genetic alteration. Additionally, under severe hypoxia, HIF-1α has different regulated mechanism in two GBM cell lines varying in their p53 status.