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The Role of Apoptosis and Autophagy in Pathogenesis of Spinalcerebellar Ataxia Type 2

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Backgrounds:

Spinocerebellar ataxia type 2 (SCA2), one of the most common ataxias worldwide, is caused by the expansion of a CAG triplet repeat located in the N-terminal coding region of the *ATXN2* gene. Alleles of the *ATXN2* gene that carry 13–31 CAG-trinucleotide repeats are present in normal individuals. Contrariwise, alleles with a CAG triplet repeat number of >31 and up to approximately 200 are present in patients with SCA2. However, mutant Ataxin-2 presents abnormal folding that gives rise to the formation of aggregates, which might trigger a series of events that lead to programmed cell death. Although the detail mechanism of pathogenesis is yet to be defined, neurotoxin, especially reactive oxygen species (ROS), released from aggregated mutant proteins, may play a role in the pathogenic process.

Materials and Methods:

In this study, the lymphoblastoid cell lines (LCLs) isolated from SCA2 patients were utilized to compare with the wild-type lymphoblastoid cells. Cell cycle and apoptosis were measured using floe cytometry assay, immunoblot. Autophagy was characterized by the increase of Atg8 (LC3 II) and the formation of acidic vesicular organelles (AVOs).

Results:

To investigate whether the mutant Ataxin-2 accumulation is caused by molecular chaperone dysfunction, heat shock induction strategy was employed and then analyzed by western blot. Interestingly, results found the autophagy marker protein, LC3 II were higher in SCA2 patients. Electron micrographs showed that only the cells expressing expanded Ataxin-2 contained aggregated protein and autophagic vacuoles.

Conclusion:

Based on the above observations we hypothesized that the aggregated mutant Ataxin-2 proteins may generate ROS in mitochondria, which subsequently up-regulate LC3 II expression levels and ultimately lead to autophagy and cell death.

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Using Heat-shook-stress-responsive Cells in Brain to Study Translational Inhibition

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Backgrounds:

When cells encounter endoplasmic reticulum (ER) stress which cause by an accumulation of misfolded proteins in the ER, unfolded protein response (UPR) well regulate the expression level of C/EBP homologous protein (CHOP) which play an important role in cell survival or apoptosis. It has been reported the uORF sequence located in 5'UTR of human chop gene (huORFchop) inhibits the rate of chop translation. However, underlying molecular mechanisms is still unknown and there is still no in vivo animal model available.

Materials & Methods:

To study the mechanism of huORFchop mediated translational control in vivo, we use the zebrafish transgenic line, termed huORFZ, harboring a construct in which the uORFchop sequence is added to the leader of GFP and is driven by a cytomegalovirus promoter. The GFP appeared only when huORFZ embryos were treated with ER stress.

Results:

Through using the heat-shock to induce ER stress, the number of GFP cells in the 72 hpf huORFZ embryo brain was depending on the heat-treated time and it was also known as the dose-dependent effect. Immunohistochemistry showed that these heat-induced GFP+ cells with longer process were GS+ glia cells, but not HuC/D+ neurons, suggesting there was some difference between GS+ glial cells and neurons, and huORF^{chop} mediated translational inhibition was only repressed by heat shock in GS+ glia cells. These indicated that brain tissue responds to heat-shock in a cell-type specific manner. Interestingly, the GFP signal first appeared in Ventricular zone of brain and TUNEL assay identified these brain GFP+ cells as non-apoptotic cells. BrdU assay showed that GFP+ cells proliferated in 24 hr after heat shook. Moreover, Lineage tracing by heat-induced GFP showed a few GFP+ cells had neurons-specific HuC/D marker in 9 dpf embryo. Now, we are developing a Laser capture Microdissection to harvest brain neurons and GFP+ cells, and using microarray analysis to find out which factors are involve in regulating huORFchop.

Conclusion:

Based on the above results, we demonstrated that repression of huORFchop mediated translational inhibition have cell-type-specific response to heat shook. These heat-induced brain GFP+ cells are GS+ redial glia-like cells which may have proliferational and deffriancational potential. Therefore, we suggested that stress-induced factors involved in huORFchop mediated translational control under heat-shock stress.

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The role of tumor associated macrophage on drug resistant in non-small cell lung cancer

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Backgrounds:

Increased evidence suggests that chronic inflammation is associated with cancer development, metastasis, invasion and drug resistant. The possible mechanisms by which inflammation can contribute to carcinogenesis include lymphocytes infiltration, cytokines and chemokines secretion, tissue remodeling and angiogenesis, etc. The inflammatory mediators facilitate the communication between tumor cells and tumor-associated host stromal tissue, thereby accelerating tumor progression. Tumor-associated macrophages (TAM) are key regulators of the link between inflammation and cancer. Previous studies found macrophage in non-small cell lung cancer (NSCLC) specimens by immunohistochemistry staining, and also discovered interplay of cancer cells and macrophages would induce secretion of certain cytokines and chemokines(MCP-1, IL-6, IL-8, IL-10, and TNF-a). However, the mechanism of cytokines and chemokines involved with tumor drug resistant in NSCLC is still obscure. It is also unclear the regulatory mechanisms in change of macrophages from M1 to M2 phase affected by tumor cells.

Materials and Methods:

we attempt to investigate the roles of TAM-mediated molecules using different characteristics of NSCLC cell lines (H2126, CL1-1, H1437, H23, H838, CL1-5, and H2009) as the study model. Furthermore, we used both cytokine antibody array to assay the secretion characteristics of cytokines. cDNA microarray was used to analyze gene expression during treatment.

Results:

We found macrophage-cultuered medium enhances chemoresistance rate of cisplatin and gemcitabine after treatment in NSCLC cell line. Following, We found 221 genes upregulation and 186 genes downregulation in which associated with adhesion molecule, inflammation cytokines and proliferation using microarray analysis after treatment in NSCLC cell line. Therefore, we used cytokine array, RT-PCR and Flow Cytometry to confirm those data. Consequently, we also found that macrophage-cultured medium could reduce cell viability through arresting cell cycle.

Conclusion:

These results suggested that macrophage-cultured medium regulate cytokines and chemokines to arrest cell cycle for chemoresistance enhancement.

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EZH2-mediate Regulation of Cancer-cell Migration

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Background:

EZH2 is a catalytic subunit of Polycomb repressive complex 2 (PRC2), which trimethylates histone H3 on lysine 27, resulting in repressing gene transcription. It has been known that EZH2 plays a pivotal role in cancer progression and metastasis. Previously we have demonstrated that EZH2 can be phosphorylated by cyclin-dependent kinase 1 (CDK1) at Thr487. The phosphorylation of EZH2 at Thr487 disrupts EZH2 binding with other PRC2 components SUZ12 and EED, and thereby inhibits its methyltransferase activity. [supported by NSC99-2632-B-039-001-MY3 and NSC101-2325-B-039-002].

Materials and Methods:

Overexpression wild-type EZH2 and mutant EZH2 in breast cancer cell line, MCF7. Transwell chamber were used in migration and invasion assay.

Result:

Moreover we found that cells expressing EZH2 T487A mutant showed higher migration and invasion ability than those expressing wild-type EZH2. In this study, we further explore the role of phosphorylation of EZH2 at Thr487 in cancer cell migration and invasion.

Conculsion:

Phosphorylation of EZH2 at Thr487 enhanced cancer cells migration and invasion ability.