Title: Gamma radiation induced endoplasmic reticulum stress downregulates WFS1, Nectin 3 and Sostdc1 gene expression in mice hippocampus

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Gamma radiation induced endoplasmic reticulum stress downregulates WFS1, Nectin 3 and Sostdc1 gene expression in mice hippocampus

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Running title:IR mediated ER stress in hippocampus

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Abstract

Introduction: Neurogenesis mainly occurs in hippocampus and is known to be sensitive to radiation. More histological changes are reported at a higher dose of radiation, while low dose radiation cause cognitive dysfunction in adult mammals. In the present study, we tried to correlate the Endoplasmic Reticulum (ER) stress mediated hippocampus dysfunction post whole body gamma radiation exposure in mice.

Methods: Mice were exposed to series of gamma radiation dose, followed by isolation of hippocampus. To elucidate the gene expression profile qPCR were performed for ER stress markers CHOP, BiP and hippocampal specific gene WFS1, Nectin3 and Sostdc1 on the isolated hippocampus. Expression of CHOP and ERK ½ were analysed by western blot on exposure to gamma radiation.

Results: qPCR results showed a significant increase in expression of ER stress specific genes CHOP, BiP and decrease in hippocampal specific genes WFS1, Nectin3 and Sostdc1. Western blot study suggests the significant increase in ER stress protein like CHOP and ERK ½ expression.

Conclusion: Exposure to gamma radiation dose significantly increased the expression of ER stress genes, suggesting ER stress plays a major role in inducing radiation mediated dysfunction of hippocampus. Also, significant downregulation of WFS 1, Nectin3 and Sostdc1 gene suggests radiation mediated effect of hippocampal CA1, CA2 and CA3 regions. A further significant increase of ERK ½ shows involvement of ERK pathway in mediating radiation induced ER stress dysfunction in mice hippocampus. Present finding may lead to identification of ER stress as a new marker to study radiation induced neurodegenerative disorder.

Keywords: Gamma radiation, Hippocampus, ER stress gene, WFS 1, Nectin3, Sostdc1

Introduction

In various clinical conditions the brain gets exposed to ionizing radiation. Though radiotherapy is considered as one of the major treatment modality in various pathological conditions the potential injury to normal tissue is unavoidable. Radiation exposure often brings with it wide array of cognitive dysfunction in adult and paediatric tumour patients (Merchant et al., 2012).Ionizing Radiation (IR)has a wide plethora of effects in both young as well as adult brain. More histological changes are reported to be observed at higher dose of radiation, while lose dose radiation causescognitive dysfunction in adult mammals (Raber et al., 2004). Repeated exposure to various stress leads to the adverse effect in cognition in various life stages (Lupien et al., 2009).Hippocampus is considered to be one of the major sites for active neurogenesis,(Praag et al., 2007;Mizumatsu., 2003).Hippocampal CA1, CA2, CA3 plays a major role in maintaining CNS homeostasis and is involved in various physiological processes (Frederick et al., 2014; Jensen et al., 1996; Dudek et al., 2016; Meyer et al., 2014).Nectrin-3 an immunoglobulin like cell adhesion molecule which is majorly localized in the CA3 pyramidal neurons plays important role in synaptic formation, maintenance and remodelling (Mizoguchiet al., 2002; Honda et al., 2006; Thompson et al., 2008).Studies showed exposure of ionizing radiation could induce ultrastructural modifications in the ER(Boraks et al., 2008). ER forms the major protein folding machinery in the cell. Cell homeostasis gets disrupted when the load of the unfolded protein increases and Unfolded Protein Response (UPR) pathway fails the repair the mis-folded protein which leads to the accumulation of those proteins in the ER lumen (Yoshida. 2007). Accumulation of this misfolded protein has been proved to cause apoptosis (Oakes et al., 2015) which ultimately leads to neurodegenerative diseases. The UPR pathway is normally active as self-defence machinery in cell, which increases the secretion of molecular chaperon such as BiP and GRP 78 which belongs to the Heat shock protein family and foldases. However when mis-folded protein accumulate in excessive amount they may overwhelm the quality control machinery. The mammalian UPR directs the cell to an apoptotic pathway (Nakagawa et al., 2000) leading to cell death. C/EBP homologous protein (CHOP), also known as GADD153 (growth arrest- and DNA damage-inducible gene 153), is triggered by ER stress. CHOP overexpression triggers cell cycle arrest and apoptosis, down-regulates the pro-survival molecule Bcl-2, and promotes the production of reactive oxygen species (Marciniaket al., 2004; Mccullough et al., 2001). On the contrary overexpression of the ER chaperone BiP reduces CHOP induction associated with ER stress and attenuates apoptosis (Wang et al., 2013).

Another player WFS1 a transmembrane protein present in the ER is shown to play a major role in mitigating ER stress response in cell (Takeda et al., 2001). Wolfram syndrome a genetic condition of diabetes, optic atrophy neurodegeneration, and psychiatric illness is reported to be caused by mutation of WFS1 gene (Stromet al., 1998;Inoue et al., 1998).Reports also suggest increase level of ER stress signalling leads to cell death, causing neuronal dysfunction in the Wolfram syndrome (Yamadaet al., 2006;Riggs et al., 2005; Kakiuchi et al., 2006).Sclerostin domain containing 1 (Sostdc1) belongs to a bone morphogenetic protein (BMP) antagonist. In the development of cultured sympathetic and cerebellar neurons, BMP family of proteins are known to play a significant role by inducing synaptogenesis and dendritic growth (Lein et al., 2002).An altered level of Sostdc1 has also been linked in various disease conditions (Park et al., 2009). Sostdc1 gene has also been reported in

thapsigargin induced ER stress in mouse osteoblasts (Hamamura et al., 2008) however mechanism is still not well understood. Cell adhesion molecules (CAM) are the major constituent of synapses and also are the modulators of synaptic activity and plasticity (Shapiro et al., 2007).Nectin-3 is a class of immunoglobulin like CAM present in both postsynaptic and presynaptic and are connected to the actin cytoskeleton via L-afadin. The nectin-afidin complex coordinates with the cadherin-catenin junction and participates in synaptic formation, remodelling and maintenance (Mizoguchiet al., 2002; Honda et al., 2006). Evidences also suggest impaired nectin mediated damage in hippocampal development and mental retardation (Park et al., 2009). Nectin-3 is shown to be abundantly present in CA3 region of hippocampus (Thompson et al., 2008)andis vulnerable to acute and chronic stress (Mizoguchi et al., 2002; Suzuki et al., 2000). For the cell to maintain homeostasis and perform its function, protein kinases plays a crucial role in various signalling networks. Mitogen activated protein kinase (MAPKs) has a conserved function and has been linked with various hippocampus mediated neurodegenerative diseases (Giovannini et al., 2008). Extracellular-signal-regulated kinase ½ (ERK1/2) is one of the member of MAPK family and has been associated and studied in various disease condition. Ultraviolet irradiation has been reported to activate ERK ½ in various primary immortalized and transformed cells (Tang et al., 2002).However radiation induced changesalteration in hippocampal Nectrin-3, WFS1 and Sostdc1 gene expression in ER stress condition via ERK ½ pathway is still unclear. To the best of our knowledge there exists no report on how radiation inducesER stress mediated alteration in hippocampus of mice exposed to whole body radiation. In the present study we have tried to understand the ER stress mediated changes in mice hippocampus after exposing to whole body gamma radiation.

Materials and Methods

Subjects

Adult Swiss albino mice (*Mus musculus*) were housed in pairs under standard laboratory conditions with artificial 12h light/dark cycle at an ambient temperature of 25–27 °C with free access to food and water. All experiments were conducted in accordance with the ethical guidelines by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India and cleared by the Institutional Animal Ethics Committee.

Gamma Irradiation

For irradiating samples ^{60}Co-gamma chamber-1200 supplied by Board of Radiation and Isotope Technology (BRIT), DAE, Mumbai was used in Centre for Application of Radiation and Radioisotope Technology (CAART, Mangalore University).The dose rate of above the gamma chamber measured and found 10.2333Gy/min using Fricke dosimetry system (Nairy et al., 2016). For the experiment 6-8 weeks age matched and weighing 25 ± 2 g male swiss albino mice (Mus musculus) were used. All the animals were supplied with standard mice feed and water ad libitum. As Jagetia et al (2003) reported 6-12 Gy of gamma radiation significantly increase lipid per-oxidation and depletion of GSH in mice exposed to whole body radiation. In the present study, mice were

exposed to gamma radiation in dose range of 7Gy, 8Gy, 9Gy, 10Gy in a well ventilated restrained perplex box. After exposure the animals were kept for 24hours and sacrificed and the hippocampus was isolated for further analysis. The above experimental protocol was approved by Institutional animal ethics committee (IAEC) of Mangalore University.

Isolation of Hippocampus

After sacrifice brains were immediately dissected out on ice and placed in a pre-chilled stereotaxic brain block (Kopf, USA). The 1mm thick sections of hippocampus were serially cut out using Paxinos and Watson atlas. The parts of same sections of the hippocampus are stored in RNA later solution (Invitrogen) for gene expression studies and in RIPA buffer (Himedia) for protein expression studies.For both Real Time qPCR and western blot analysis "n=5"adult Swiss albino mice were kept in each experimental group.

Real Time qPCR analysis

Total RNA isolated from cells by Trizol reagent (Invitrogen).The Qubit RNA assay kit (Invitrogen) was used for quantifying the isolated RNA. 1µg of the total RNA was used for a 20µl reaction. The Verso cDNA synthesis kit (Applied Biosystems) was used for the reverse transcription (RT) reaction. For the quantitative RT-PCR, SYBR Select Master Mix (Applied Biosystems) was used in QuantStudio12K (Life Technology) real-time PCR machine with primers (Table 1) specific to detect the target messenger RNA (mRNA).

Western Blot

The western blot analysis was carried out to understand the expression level of CHOP and ERK pathway on exposure to IR in mice hippocampus. The tissues were lysed using lysis buffer (Himedia) and stored at -20 °C until for further analysis. Qubit protein assay kit (Invitrogen) in Qubit 2.0 fluorometer (Invitrogen) was used to quantify the isolated protein from the hippocampus tissue homogenate. The 40 µg of the quantified protein sample was resolved in 10% SDS polyacrylamide gel and further transferred to nitrocellulose membrane. After transfer, the membrane was blocked using 3% BSA in Tris-buffered saline and Tween 20 mixture (0.2 %) and incubated in primary antibody overnight at 4°C. The primary antibodies- anti-GAPDH (1:1000, Abchem), anti-CHOP (1:1000, Cell signalling), anti-ERK 1/2 (1:1000, Pierce)were used. The Bands were visualized in ChemiDoc (Bio rad) using corresponding horseradish peroxidase conjugated secondary antibodies (Sigma). The bands were quantified using imageJ software and graphs were plotted by Graphpad prism-3 software.

Statistical analysis

Statistical analysis was performed using Prism 3 software (GraphPad Software Inc.) and performed one way ANOVA followed by Dunnett's multiple range test. The data were expressed as mean ± standard error (SE). The $p<0.05$ was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Results

Effect of Radiation on ER stress and hippocampus specific gene expression

BiP and CHOP are well-known ER chaperones and get up-regulated under conditions of ER stress. We assessed the expression of BiP, CHOP, WFS1, Sostdc1 and nectin3 by quantitative RT-qPCR on irradiated mice hippocampus. Result demonstrate increase in CHOP and BiP gene expression with increase in dose of 7Gy to 10Gy and also4 fold increase of ER stress specific gene CHOP (***P<0.001) and 6fold increase inBiP (**P<0.01) at 10Gy of gamma radiation dose with respect to control (Fig. 1 a-b).Further, 7-10 Gy dose of gamma radiation significantly downregulates the expression of nectin3 gene (***P<0.001), WFS1 (***P<0.001) and Sostdc1 (***P<0.001)by 1 fold which ultimately at the 10Gy dose(Fig. 2a-c).

Protein expression analysis of CHOP and ERK ½

CHOP gene has been majorly used as hallmark for the ER stress mediated apoptosis and induction of this gene confirms ER stress mediated apoptosis. Whole body gamma radiation dose significantly increased (**P<0.01) expression of CHOP (Fig. 3 a-b) protein level suggesting induction of ER stress mediated apoptosis in the isolated hippocampus. Significant increase in the CHOP protein expression (**p<0.01) at 10Gy of dose gamma advocates profuse ER stress mediated apoptosis.Also significant (*P<0.05)increase of ERK ½protein expression at the dose range of 10Gy dose of radiation are observed on hippocampus (Fig. 3 a,c) which further validate the involvement of ERK ½ pathway in inducing ER stress mediated apoptosis in isolated hippocampus. Significant increase in the levels of CHOP and ERK ½ suggest IR induces hippocampal dysfunction by inducing ER stress mediated apoptosis which might be further responsible for various IR mediated hippocampal dysfunction.

Discussion

Understanding the molecular machinery responsible for radiation induced cognitive dysfunction will provide insight into the molecular mechanism and neurobiology of stress induced neurodegenerative disorders. Here we tried to understand how radiation onsets ER stress induced dysfunction of hippocampus of mice brain post whole body radiation. We observed a positive correlation with the upregulation of ER stress specific genes and also the genes responsible for various physiological functions in the hippocampal regions. ER stress has been reported to disrupt neuronal functions and is responsible for various neurological disorders like Alzheimer's disease, Parkinson's disease and Huntington's disease (Siman et al., 2001).Radiation induced ER stress has also been reported by us and others in various cell lines (Zhang et al., 2001). In the present study we have found elevated expression of GRP78/BiPwhich has been reported earlier to have dual role which activates under ER stress condition as self defence mechanism in the cell, but under unresolved ER stress this leads to cell death (Akutsu et al., 2007). Another marker CHOP a downstream component of ER stress pathway is reported to get upregulated along with the induction of BiP signalling and lead the cell towards apoptotic pathway. CHOP over expression has been linked with various neurodegenerative diseases and also targeted for development of

therapeutic drugs against ER stress (Ohoka et al., 2005).In the present study we observed significant upregulation with the increasing gamma radiation dose suggesting the 7Gy gamma radiation exposure brings about significant induction of ER stress mediated apoptosis in mice hippocampus.

Previous reports have suggested cells deficient in WFS1 are more susceptible to ER stress mediated apoptosis. Present study we have observed reduction of WFS1 gene with the increase of radiation dose in sync with increase of BiP and CHOP suggesting WFS1 plays an important role in mediating ER stress mediated apoptosis. Also reduced level of WFS1 is linked with a genetic condition leading to wolfram syndrome which causes severe depression, psychosis, or organic brain syndrome, as well as impulsive verbal and physical aggression (Takeda et al., 2001).In the current study we observed a decrease in WFS1 gene expression with increase of radiation dose suggesting a link role of WFS1gene in inducing ER stress mediated dysfunction in hippocampus.

Previous reports have suggested CAM nectin-3 is plays major role in hippocampal dependent learning and memory (Wang et al., 2013).Reduced level of nectin have been associated with early life stress, disruption of synaptic contacts and also hampered spatial memory. In our present study we observed a dose dependent decrease of nectin-3 expression suggesting 7Gy to 10Gy gamma radiation have severe effect in destabilizing the hippocampal neurons which might be disrupting hippocampal dependent cognitive functions.Downregulation of nectin3 also suggest hampered episodic memories and loss of neurogenesis in CA3 region of hippocampus.

Sostdc1 gene which is predominantly found in CA2 region of hippocampus is also reported to play a major role in Wnt receptor signalling pathway (Inestrosa et al., 2005). We have observed significant downregulation of Sostdc1 gene on exposure of gamma radiation which suggest radiation causes significant changes in the wnt signalling pathway and this might ultimately leading to various hippocampus induced neuro degenerative diseases.

Heat shock protein is said to activate various kinase pathways that control proliferation and survival like ERK1/2, Akt (Mebratu and Tesfaigzi, 2009). ERK1/2 activation is reported to promote ER stress induced cell death neuroblastoma cell line(Arai et al., 2004;Mukerjee et al., 2000).The Suppression of ERK ½ or Akt activation during stress condition increases heat sensitivity; on the contrary overexpression of wild-type ERK ½ protects cells from stress (Gabai et al., 2000).In the present study we have observed significant upregulation of ERK ½ protein expression post 10Gy exposure of gamma radiation suggesting ERK pathway mediated cell death which might be causing via phosphorylation of pro-apoptotic signal of DAPK as also reported earlier by (Mebratu et al., 2009) (Fig.4).

In conclusion our present study suggests a 7Gy dose of whole body gamma radiation in mice is sufficient to induce ER stress specific markers BiP and CHOP and also downregulates hippocampal genes WFS1, Sostdc1, Nectrin3 which ultimately disrupt the hippocampal homeostasis. Significant increase of ERK ½ also suggests cells innate response to overcome ER stress. As hippocampus is involved in wide array of physiological functions, radiation induced damage in the hippocampus might lead to various neurodegenerative diseases such as AD, PD, etc. Present study may lead to identification ofER stress and hippocampal genes as new markers to study radiation induced neurodegenerative disorder induced by hippocampal dysfunction.

The authors declare that they have no conflict of interest.

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References

Abayomi O (1996) Pathogenesis of irradiation-induced cognitive dysfunction. ActaOncol (Madr) 35:659–63. doi: 10.3109/02841869609083995

Arai K, Lee S-R, van Leyen K, et al (2004) Involvement of ERK MAP kinase in endoplasmic reticulum stress in SH-SY5Y human neuroblastoma cells. J Neurochem 89:232–239. doi: 10.1111/j.1471-4159.2004.02317.x

Chen Y, Dube CM, Rice CJ, Baram TZ (2008) Rapid Loss of Dendritic Spines after Stress Involves Derangement of Spine Dynamics by Corticotropin-Releasing Hormone. J Neurosci 28:2903–2911. doi: 10.1523/JNEUROSCI.0225-08.2008

Conrad CD (2006) What is the functional significance of chronic stress-induced CA3 dendritic retraction within the hippocampus? BehavCognNeurosci Rev 5:41–60. doi: 10.1177/1534582306289043

Dudek SM, Alexander GM, Farris S (2016) Rediscovering area CA2: Unique properties and functions. Nat Rev Neurosci 17:89–102. doi: 10.1038/nrn.2015.22

Frederick L Hitti, Siegelbaum SA (2014) The hippocampal CA2 region is essential for social memory Frederick. Nature 508:88–92. doi: 10.1038/nature13028.The

Gabai VL, Yaglom JA, Volloch V, et al (2000) Hsp72-mediated suppression of c-Jun N-terminal kinase is implicated in development of tolerance to caspase-independent cell death. Mol Cell Biol 20:6826–6836. doi: 10.1128/MCB.20.18.6826-6836.2000

Giovannini MG, Cerbai F, Bellucci A, et al (2008) Differential activation of mitogen-activated protein kinase signalling pathways in the hippocampus of CRND8 transgenic mouse, a model of Alzheimer's disease. Neuroscience 153:618–633. doi: 10.1016/j.neuroscience.2008.02.061

Hamamura K, Liu Y, Yokota H (2008) Microarray analysis of thapsigargin - Induced stress to the endoplasmic reticulum of mouse osteoblasts. J Bone Miner Metab 26:231–240. doi: 10.1007/s00774-007-0825-1

Honda T, Sakisaka T, Yamada T, et al (2006) Involvement of nectins in the formation of puncta adherentia junctions and the mossy fiber trajectory in the mouse hippocampus. Mol Cell Neurosci 31:315–325. doi: 10.1016/j.mcn.2005.10.002

Inestrosa NC, Godoy JA, Quintanilla RA, et al (2005) Peroxisome proliferator-activated receptor γ is expressed in hippocampal neurons and its activation prevents β-amyloid neurodegeneration: Role of Wntsignaling. Exp Cell Res 304:91–104. doi: 10.1016/j.yexcr.2004.09.032

Inoue H, Tanizawa Y, Wasson J, et al (1998) A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). Nat Genet 20:143–8. doi: 10.1038/2441

Jagetia GC, Baliga MS, Venkatesh P, Ulloor JN (2003) Influence of Ginger Rhizome (ZingiberofficinaleRosc) on Survival, Glutathione and Lipid Peroxidation in Mice After Whole-Body Exposure to Gamma Radiation. Radiat Res 160:584–592. doi: 10.1667/RR3057

Jensen O, Lisman JE (1996) Hippocampal CA3 region predicts memory sequences: accounting for the phase precession of place cells. Learn Mem 3:279–287. doi: 10.1101/lm.3.2-3.279

Kakiuchi C, Ishiwata M, Hayashi A, Kato T (2006) XBP1 induces WFS1 through an endoplasmic reticulum stress response element-like motif in SH-SY5Y cells. J Neurochem 97:545–555. doi: 10.1111/j.1471-4159.2006.03772.x

KIM J-S, LEE H-J, KIM JC, et al (2008) Transient Impairment of Hippocampus-dependent Learning and Memory in Relatively Low-Dose of Acute Radiation Syndrome is Associated with Inhibition of Hippocampal Neurogenesis. J Radiat Res 49:517–526. doi: 10.1269/jrr.08020

Lein PJ, Beck HN, Chandrasekaran V, et al (2002) Glia induce dendritic growth in cultured sympathetic neurons by modulating the balance between bone morphogenetic proteins (BMPs) and BMP antagonists. J Neurosci 22:10377–10387.

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 10:434–445. doi: 10.1038/nrn2639

Mebratu Y, Tesfaigzi Y (2009) How ERK1/2 Activation Controls Cell Proliferation and Cell Death Is Subcellular Localization the Answer? Cell Cycle 8:1168–1175. doi: 10.1146/annurev.biophys.37.032807

Merchant TE, Pollack IF, Loeffler JS (2012) Brain tumors across the age spectrum: biology, therapy, and Late Effects. SeminRadiatOncology 20:58–66. doi: 10.1016/j.semradonc.2009.09.005.Brain

Meyer MA (2014) Highly expressed genes within hippocampal sector CA1: Implications for the physiology of memory. NeurolInt 6:32–35. doi: 10.4081/ni.2014.5388

Mizoguchi A, Nakanishi H, Kimura K, et al (2002) Nectin: An adhesion molecule involved in formation of synapses. J Cell Biol 156:555–565. doi: 10.1083/jcb.200103113

Mizumatsu S, Monje ML, Morhardt DR, et al (2003) Extreme Sensitivity of Adult Neurogenesis to Low Doses of Extreme Sensitivity of Adult Neurogenesis to Low Doses of X-Irradiation. Cancer Res 63:4021–4027.

Mukerjee N, McGinnis KM, Park YH, et al (2000) Caspase-mediated proteolytic activation of calcineurin in thapsigargin-mediated apoptosis in SH-SY5Y neuroblastoma cells. Arch BiochemBiophys 379:337–43. doi: 10.1006/abbi.2000.1889

Nairy RK, Bhat NN, Sanjeev G, Yerol N (2016) Dose-Response Study Using Micronucleus Cytome Assay: A Tool ForBiodosimetry Application. RadiatProt Dosimetry ncw045. doi: 10.1093/rpd/ncw045

Park J-W, Park E-S, Choi EN, et al (2009) Altered brain gene expression profiles associated with the pathogenesis of phenylketonuria in a mouse model. ClinChimActa 401:90–9. doi: 10.1016/j.cca.2008.11.019

Raber J, Rola R, LeFevour A, et al (2004) Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. Radiat Res 162:39–47. doi: 10.1667/RR3206

Riggs AC, Bernal-Mizrachi E, Ohsugi M, et al (2005) Mice conditionally lacking the Wolfram gene in pancreatic islet beta cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. Diabetologia 48:2313–2321. doi: 10.1007/s00125-005-1947-4

Rola R, Raber J, Rizk A, et al (2004) Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. ExpNeurol 188:316–330. doi: 10.1016/j.expneurol.2004.05.005

Seki T, Namba T, Mochizuki H, Onodera M (2007) Clustering, migration, and neurite formation of neural precursor cells in the adult rat hippocampus. J Comp Neurol 502:275–290. doi: 10.1002/cne

Shapiro L, Love J, Colman DR (2007) Adhesion Molecules in the Nervous System: Structural Insights into Function and Diversity. Annu Rev Neurosci 30:451–474. doi: 10.1146/annurev.neuro.29.051605.113034

Strom TM, Hörtnagel K, Hofmann S, et al (1998) Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. Hum Mol Genet 7:2021–8. doi: 10.1093/hmg/7.13.2021

Suzuki K, Hu D, Bustos T, et al (2000) Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. Nat Genet 25:427–430. doi: 10.1038/78119

Swift RG, Sadler DB, Swift M (1990) Psychiatric findings in Wolfram syndrome homozygotes. Lancet 336:667–669. doi: 10.1016/0140-6736(90)92157-D

Takeda K, Inoue H, Tanizawa Y, et al (2001) WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. Hum Mol Genet 10:477–484. doi: 10.1093/hmg/10.5.477

Tang D, Wu D, Hirao A, et al (2002) ERK activation mediates cell cycle arrest and apoptosis after DNA damage independently of p53. J BiolChem 277:12710–12717. doi: 10.1074/jbc.M111598200

Thompson CL, Pathak SD, Jeromin A, et al (2008) Genomic Anatomy of the Hippocampus. Neuron 60:1010–1021. doi: 10.1016/j.neuron.2008.12.008

Van Praag H (2005) Exercise Enhances Learning and Hippocampal Neurogenesis in Aged Mice. J Neurosci 25:8680–8685. doi: 10.1523/JNEUROSCI.1731-05.2005

Wang XD, Su YA, Wagner K V., et al (2013) Nectin-3 links CRHR1 signaling to stress-induced memory deficits and spine loss. Nat Neurosci 16:706–713. doi: 10.1038/nn.3395

Whitmarsh AJ, Kuan CY, Kennedy NJ, et al (2001) Requirement of the JIP1 scaffold protein for stress-induced JNK activation. Genes Dev 15:2421–2432. doi: 10.1101/gad.922801

Yamada T, Ishihara H, Tamura A, et al (2006) WFS1-deficiency increases endoplasmic reticulum stress, impairs cell cycle progression and triggers the apoptotic pathway specifically in pancreatic β-cells. Hum Mol Genet 15:1600–1609. doi: 10.1093/hmg/ddl081

Figure legends

Fig.1: qRT-PCR analysis of isolated hippocampus on exposure to post whole body gamma radiation of mice. The qRT- PCR analysis of the transcript levels of ER stress specific gene CHOP (a) and BiP (b) on exposure to 7Gy, 8Gy, 9Gy and 10Gy. The GAPDH is used as internal control for the estimation of target gene expression. For quantitative representation graph is plotted as gene expression compared to control. One way ANOVA followed by Dunnett's multiple range were then used to check the significance based on control. Error bars represent Mean ± SE with ANOVA parameters (*p<0.05;**p<0.01; ***p<0.001).

Fig.2: qRT- PCR analysis of the transcript levels of nectin3 (a), WFS1 (b) and SOSTDC (c). Graph is plotted of gene expression relative to control and 7Gy, 8Gy, 9Gy and 10Gy dose of gamma radiation. GAPDH served as an internal control. To evaluate the significant fold induction as compared to control one way ANOVA and Dunnett's multiple range test was performed (*P<0.05;**P<0.01; ***P<0.001). Error bars represent Mean ± SE.

Fig.3: Western blot analysis of protein levels of hippocampus post whole body gamma radiation. CHOP and ERK ½ protein level was analysed. Representative western blot images (3a).Quantitative analysis of western blot of CHOP (3b) and ERK ½ (3c). Quantitative graphical representation was done using Graphpad prism software. To represent a statistically significant protein expression as compared to control one way ANOVA and Dunnett's multiple range test was performed with the one way ANOVA parameters (*P<0.05;**P<0.01; ***P<0.001). Error bars are the representation of Mean ± SE.

Fig.4: Hypothetical schematic diagram showing mechanism of whole body mice gamma radiation induced ER stress mediated disruption of hippocampal homeostasis. Radiation induces inhibition of Sostdc1 gene resulting in disruption of wnt pathway which might me leading cells to ER stress. Also radiation downregulates Nectin3 resulting in hampered nectinafidin complex ultimately might be causing various neuro-degenerative disorders. Onset of ER stress leads to the dissociation of BIP from the unfolded protein ultimately leading to the sequential activation of IRE1α, PERK and AT6 pathway downstream in the ER lumen. Radiation inhibits wfs1 gene expression resulting in IRE1α mediated apoptosis. IRE1α also activated MEK which leads to the phosphorylation of ERK1/2 which ultimately leads to apoptosis via phosphorylating DAPK.