Physiopathology of Radiation-Induced Neurotoxicity

John R. Fike, Ph.D.
Brain and Spinal Injury Center
University of California, San Francisco
Clinical Radiation Exposure of the Brain

- ~ 1,400,000 new cancer cases are expected in the US/year
  - Includes primary brain tumors (~ 20,000);
- Brain metastases will occur in 20-40% of these patients;
- Currently ~ 200,000 patients/year receive large field or whole brain irradiation.
Radiation Brain Injury After ‘Higher Doses’

• Generally restricted to white matter;
• Generally a late effect, appearing after a latent period;
• Imaging and clinical changes;
• Histology: demyelination, vascular damage, necrosis.
‘Low Dose’ Radiation Brain Injury: Cognitive Impairment

- Progressive cognitive impairment occurs in up to 50% of long-term brain tumor survivors (> 6 months);
- No obvious imaging/tissue changes;
- Currently untreatable;
- Unknown pathogenesis but hippocampus is often involved.
- Understanding the factors involved may provide insight for the development of treatment strategies.
The Dogma

“In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerate.”

Degeneration and Regeneration of the Nervous System

Santiago Ramon y Cajal, 1913
Neurogenic Zones of the Mammalian Forebrain
Hippocampus

- An integral part of the temporal lobe memory system.
- An active site of neurogenesis
New Neurons are Integrated Into the Hippocampal Circuitry
Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus

Nohjin Kee1-3,5, Cátia M Teixeira1-5, Afra H Wang1,3 & Paul W Frankland1-3

Throughout adulthood, new neurons are continuously added to the dentate gyrus, a hippocampal subregion that is important in spatial learning. Whether these adult-generated granule cells become functionally integrated into memory networks is not known. We used immunohistochemical approaches to visualize the recruitment of new neurons into circuits supporting water maze memory in intact mice. We show that as new granule cells mature, they are increasingly likely to be incorporated into circuits supporting spatial memory. By the time the cells are 4 or more weeks of age, they are more likely than existing granule cells to be recruited into circuits supporting spatial memory. This preferential recruitment supports the idea that new neurons make a unique contribution to memory processing in the dentate gyrus.
Do Early Effects in the SGZ Translate into Later Changes in Neurogenesis?
Effects of X-rays on New Neuron Production in the Mouse SGZ

Mizumatsu et al, Can Res 2003
Changes in Neurogenesis are Persistent After X-Irradiation
Are Changes in Neurogenesis Associated with Functional Impairments?
Barnes Maze Performance of Control and Irradiated Mice (Hippocampal Dependent Learning)

Raber et al, Rad. Res. 2004
Changes in Neurogenesis (and Cognitive Impairment?) are Influenced by Microenvironmental Factors

Inflammation
Oxidative Stress
Activated Microglia Increase After Irradiation
Association Between New Neuron Production and Inflammation After Irradiation

[Graph showing the association between radiation dose and the percentage of doubled labeled cells and BrdU+ neurons and microglia.]

Neurons
Activated Microglia

% Doubled Labeled Cells

Radiation Dose (Gy)

% BrdU+ Neurons

% BrdU+ Microglia
Changes in Neurogenesis (and Cognitive Impairment?) are Influenced by Microenvironmental Factors

Inflammation
Oxidative Stress
Oxidative Stress, Detected by 4-Hydroxynonenal, is Increased in Mouse Brain 2 Months After 5 Gy
Irradiation Induces Oxidative Stress in Cells/Tissues

Irradiation

$O_2^{-}$

SOD-Me$^+$

$H_2O_2$

Catalase

GPx

$H_2O$

Lipid oxidation
Protein oxidation
Superoxide Dismutase

- **SOD1 (Cu/Zn SOD):** Cytosolic
- **SOD2 (MnSOD):** Mitochondrial
- **SOD3 (EC-SOD):** Extracellular
EC-SOD Deficiency Leads to Indications of Oxidative Stress

4-Hydroxynonenal

Nitrotyrosine

Lack of EC-SOD Does Not Induce Compensatory Changes in Other Antioxidant Enzymes
Lack of EC-SOD Does Not Induce Compensatory Changes in SOD1 or SOD2 Activities
SOD Deficiency and its effects on Neurogenesis in the Dentate SGZ
Hippocampal-Dependent Contextual Fear Conditioning After Irradiation (10 Gy) in WT and SOD3 Deficient Mice

Raber et al, Hippocampus 2011
Does Irradiation Disrupt Neuronal Function in the Dentate Gyrus?
Behavioral Exploration Induces Immediate Early Genes

A. Caged Control
B. Arc
C. zif268
D. cfos

Immediate Early Gene *Arc* (activity-regulated cytoskeleton-associated protein):

A molecular marker of neuronal activity during learning and memory

• *Arc* is tightly coupled to behavioral encoding of information in neuronal circuits

• *Arc* protein is required for learning and memory consolidation;

• *Arc* KO mice are cognitive impaired for hippocampal tasks;
Experimental Approach to detect *Arc* mRNA and Arc Protein

Fluorescence *in situ* hybridization (FISH) detects *Arc* mRNA

Fluorescence immunostaining detects Arc protein
**Arc** is Dynamically Regulated

*Arc* mRNA foci observed within 5 min of behavioral exploration

*Arc* mRNA translocated to the cytoplasm 8-10 min later

Arc protein detected in the cytoplasm ~30 min later

Cytoplasmic *Arc* mRNA from 1st exploration and *Arc* foci from the 2nd exploration
Arc Protein Expressed in Newly Born Neurons in the Dentate SGZ

Arc Protein = Red

BrdU = Green

NeuN = Blue
X-irradiation Reduces Arc Expression in the Dentate Gyrus

Arc mRNA

Arc Prot.

Caged Controls O Gy Behavior 10 Gy Behavior

X-Irradiation Affects the Molecular Distribution of Arc at the Level of mRNA and Protein

The % of Neurons Expressing Arc mRNA (A) and Arc Protein (B) are Correlated with Numbers of Activated Microglia After Irradiation

\[ R^2 = 0.66 \]

\[ R^2 = 0.54 \]
Summary

• Neurogenesis and $Arc$ expression are sensitive to irradiation;

• Changes in neurogenesis are associated with cognitive impairments;

• Environmental context (inflammation/oxidative stress) may be critical factors in the evolution and treatment of cognitive impairment;

• A better understanding of these processes may provide insight into new strategies to ameliorate or treat the adverse effects of brain irradiation.
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