ABSTRACT

Proteolytic cleavage of htt is regarded as a critical event in the pathogenesis of HD. Expression of htt fragments containing an expanded polyglutamine repeat are toxic in vitro and in vivo, and accumulation of N-terminal truncated products of htt are observed in human and mouse HD brain. Notably, the presence of htt fragments prior to clinical onset of HD suggests that htt cleavage may be a crucial, causal event in the pathogenesis of HD. However the relationship between specific huntingtin fragments and the pathogenesis of HD is unknown. Mutagenesis of all caspase sites in mutant huntingtin prevents toxicity in cultured cells and caspase inhibitors improve survival of neurons transfected with mutant htt. Caspase resistant (CR) htt mouse models therefore would be ideal systems in which to assess whether creation of caspase generated fragments of htt underlie the pathogenesis of HD in vivo. To examine whether a specific caspase cleavage fragment of mutant huntingtin is responsible for the selective neurodegeneration observed in HD, we generated YAC transgenic mice expressing selective mutations of the caspase cleavage sites within mutant huntingtin. We show, using sequential mutagenesis, that caspase-6 and not caspase-3, mediated cleavage of mutant htt is responsible for the HD-related behavioral phenotype and selective striatal neurodegeneration observed in the YAC128 model of HD. Activation of caspase-6 and nuclear translocation of htt fragments coincide with onset of motor dysfunction in the YAC128 model, supporting a role for a specific nuclear htt fragment in initiating neuronal dysfunction. Furthermore, caspase-6 cleavage of mutant htt influences susceptibility to excitotoxic stress highlighting caspase-6 mediated proteolysis of htt and excitotoxicity as a primary mechanism underlying motor dysfunction and neuropathology in HD. The results presented in this thesis support and further refine the toxic fragment hypothesis by identifying a specific proteolytic cleavage site in htt that is required for initiating a sequence of events which culminate in the death of selective neurons affected in HD. This evidence demonstrates that generation of a specific fragment of mutant htt in vivo represents an initiating event in the pathogenesis of HD and identifies novel approaches for inhibiting cell death in neurodegenerative disorders such as HD.

BIOGRAPHICAL NOTES

Born: September 14, 1963

Academic Studies: B.Sc. Concordia University, 1987

GRADUATE STUDIES

Field of Study: Neurodegeneration and Programmed Cell Death

Courses

| MEDG505 | Genome Analysis | Dr. P. Heltier |
| MEDG520 | Advanced Human Genetics | Dr. C. Brown |
| MEDG530 | Advanced Human Genetics | Dr. B. Simpson |
| MEDG540 | Seminar | Dr. F. Dill |
| MEDG548 | Directed Studies | Dr. C. Brown |
| BIOL530 | Biology of the Cell | Dr. D. Moerman |

AWARDS

- University Graduate Fellowship – Master’s 2000-2001
- Michael Smith Foundation for Health Research – Master’s 2001-2003
- Canadian Institute of Health Research – Doctoral 2002-2005

SELECTED PUBLICATIONS


- A YAC mouse expressing a truncated fragment of huntingtin (shortstop) displays widespread neuronal inclusions but no neuronal dysfunction, behavioral changes or degeneration. Elizabeth J. Slow and Rona K. Graham, Alexander P. Osmand, Rebecca S. Devon, Ge Lu, Yu Deng, Jacqueline Pearson, Kuljeet Vaid, Nagat Bissada, Ronald Wetzel, Blair R. Leavitt and Michael R. Hayden. PNAS 102;11402-11407
Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms.

**FULL-LENGTH HUNTINGTIN PROTEIN IS REQUIRED FOR STRIATAL SPECIFICITY OF AGGREGATES IN YAC MOUSE MODELS OF HUNTINGTON DISEASE**

Rona K. Graham, Elizabeth J. Slow, Rebecca S. Devon, Kuljeet Vaid and Michael R. Hayden
Neuroscience Conference, 2003

In vivo inhibition of caspase-6 cleavage of expanded huntingtin protects against neurodegeneration
Neuroscience, HDF, ASHG Conference (s), 2004

Links between proteolysis and neurotoxicity identify novel approaches for modifying the pathogenesis of HD.
Rona K Graham, Anat Yanai, Alaa El Husseini and Michael R Hayden
HD Congress, 2005

**SUPERVISORY COMMITTEE**

Dr. Michael Hayden, Research Supervisor (Medical Genetics)
Dr Shoukat Dedhar (Biochemistry and Molecular Biology)
Dr Lynn Raymond (Psychiatry)
Dr Wendy Robinson (Medical Genetics)
Dr Cheryl Wellington (Pathology)

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**SELECTED PRESENTATIONS**

**FULL-LENGTH HUNTINGTIN PROTEIN IS REQUIRED FOR STRIATAL SPECIFICITY OF AGGREGATES IN YAC MOUSE MODELS OF HUNTINGTON DISEASE**

Rona K. Graham, Elizabeth J. Slow, Rebecca S. Devon, Kuljeet Vaid and Michael R. Hayden
Neuroscience Conference, 2003

In vivo inhibition of caspase-6 cleavage of expanded huntingtin protects against neurodegeneration
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Dr Shoukat Dedhar (Biochemistry and Molecular Biology)
Dr Lynn Raymond (Psychiatry)
Dr Wendy Robinson (Medical Genetics)
Dr Cheryl Wellington (Pathology)

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**PROGRAMME**

The Final Oral Examination
For the Degree of

DOCTOR OF PHILOSOPHY
(Medical Genetics)

**RONA K GRAHAM**

B.Sc. Concordia University, 1987

Wednesday, January 25, 2006, 9:00 am
Room 200, Graduate Student Centre

"In vivo Characterization of Caspase Resistant Huntingtin: Insights into the Pathogenic Mechanism of Huntington Disease"

**EXAMINING COMMITTEE**

Chair:
Dr. Stelvio Bandiera (Pharmaceutical Sciences)

Supervisory Committee:
Dr. Michael Hayden, Research Supervisor (Medical Genetics)
Dr. Cheryl Wellington (Pathology)
Dr. Shoukat Dedhar (Biochemistry and Molecular Biology)

University Examiners:
Dr. Ken Baimbridge (Physiology)
Dr. Lorne Clarke (Medical Genetics)

External Examiner:
Dr. Patrik Brundin
Department of Experimental Medical Science
Wallenberg Neuroscience Center
Lund, Sweden