CURRICULUM VITAE

Dr. CLAUDIO COSTANTINI

PERSONAL INFORMATION

Date of birth:	February 24, 1976
Place of birth:	Liestal, Switzerland
Citizenship:	Italian
Languages:	Italian, English

EDUCATION

1991-1995	Liceo Scientifico G. Marconi, Via Nanterre 10, Pesaro High School Diploma of *Maturità Scientifica*
1996-2000	Degree in Biological Sciences University of Urbino, Italy Degree taken with full marks on the 12th of July 2000
2002- 2005	Research Doctorate in Molecular and Cellular Biology and Pathology (Department of Pathology, Section of General Pathology of the Medical School of the University of Verona).

RESEARCH EXPERIENCE

1999-2000:	Undergraduate Student G. Fornaini Institute of Biological Chemistry University of Urbino, Urbino, Italy Centro di Biotecnologie, University of Urbino, Fano, Italy Supervisor: Prof. M. Magnani
February 2001:	Visiting Scientist Dipartimento di Genetica e Biologia Molecolare Università La Sapienza, Rome, Italy Supervisor: Prof. A. Torroni C.I.B. fellowship
2001- 2004:	Research Fellow Department of Pathology – Section of General Pathology School of Medicine University of Verona, Italy Supervisor: Prof. F. Rossi 2001: "Consorzio per lo sviluppo degli studi universitari" fellowship 2002- 2005: Research Doctorate in Molecular and Cellular Biology and Pathology

Feb 2004-Dec 2005: Research Intern
Department of Medicine
University of Wisconsin-Madison
Madison, WI
Supervisor: Prof. L. Puglielli

2006: Research Fellow
Department of Pathology – Section of General Pathology
School of Medicine
University of Verona, Italy
Supervisor: Prof. F. Rossi

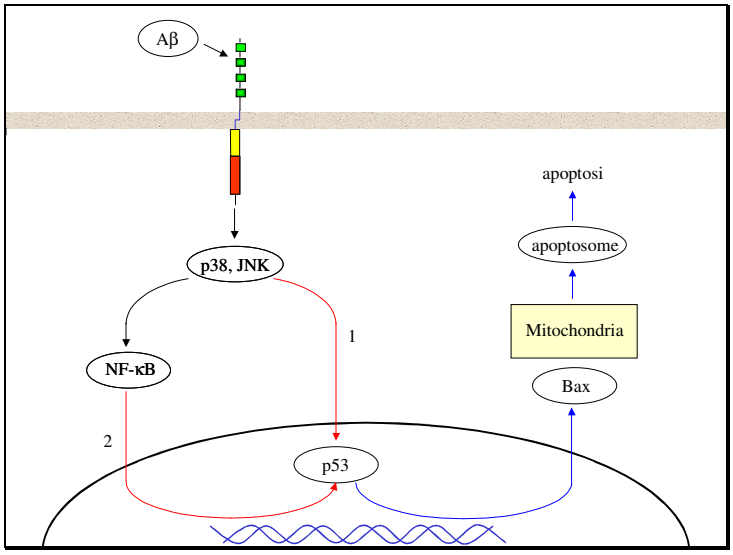
MAIN RESEARCH ACTIVITY

1) Pathogenesis of Alzheimer's disease: role of p75 neurotrophin receptor in the neurotoxicity of beta-amyloid peptides

Background:
Alzheimer's disease is a neurodegenerative disorder characterized by a progressive loss of synapses and neurons in several areas of the brain. The two pathological hallmarks of the disease are intraneuronal neurofibrillary tangles and amyloid plaques. The microtubule-associated protein tau is the main component of neurofibrillary tangles whereas beta-amyloid (Aβ) is the main constituent of the senile plaques. According to the "amyloid cascade hypothesis", an accumulation of Aβ iniziates a cascade of events that is responsible for the neuronal dysfunction and subsequent degeneration. It is believed that the neurotoxic activity of Aβ involves two different, but not mutually exclusive, mechanisms: a direct mechanism whereby Aβ interacts with neuronal membranes and thus injures neurons directly; alternatively, Aβ may damage neurons indirectly by activating microglia and astrocytes to produce toxic and inflammatory mediators. Several receptors have been involved in Aβ binding, including the neurotrophin receptor p75NTR.
Four different neurotrophins have been identified: NGF (nerve growth factor), BDNF (brain-derived growth factor), NT-3 (neurotrophin-3), NT-4/5 (neurotrophin 4/5). Their function depends on the binding to two different groups of receptors: p75NTR, that binds all the neurotrophins with a similar low affinity, and Trk receptors, that bind specific neurotrophins with high affinity. p75NTR was first functionally identified as a cooperative facilitator of the function of Trk receptors. Recent studies have made it clear that p75NTR serves several functions, separate and distinct from its interaction with the Trk receptors, including survival/apoptosis, regulation of axonal outgrowth and synaptic plasticity.
The possible involvement of p75NTR in the pathogenesis of Alzheimer's disease was suggested by the following evidence: *in vitro* experiments have shown that p75NTR can bind fibrillar Aβ and tranduce an apoptotic signalling pathway; *in vivo* observations have revealed that the cholinergic neurons of the basal forebrain, which are early and severely affected in AD, express high levels of $p75^{NTR}$, whereas the cholinergic neurons of the brainstem, which do not express $p75^{NTR}$, remain undamaged.

Results:
By using a neuroblastoma cell line devoid of neutrophin receptors (SK-N-BE) and stably transfected with full-length or various truncated forms of p75NTR, we were able to demonstrate that p75NTR is involved in the direct signalling of cell death by Aβ via the function of its death domain. Moreover, we could demonstrate that proinflammatory cytokines, such as TNFα and IL-1β, could potentiate the

neurotoxic action of Aβ mediated by p75NTR signalling (Perini G. *et al.* J Exp Med. 2002 Apr 1;195(7):907-18).
We further analyzed the signalling pathway activated by p75NTR in the presence of fibrillar Aβ (Costantini C. *et al*. J Mol Neurosci. 2005;25(2):141-56). We could demonstrate that the mitogen-activated protein kinases (MAPKs) p38 and c-Jun N-terminal kinase (JNK) were activated in a death domain-dependent manner, followed by NF-kappaB translocation and p53 activation, according to the following picture:



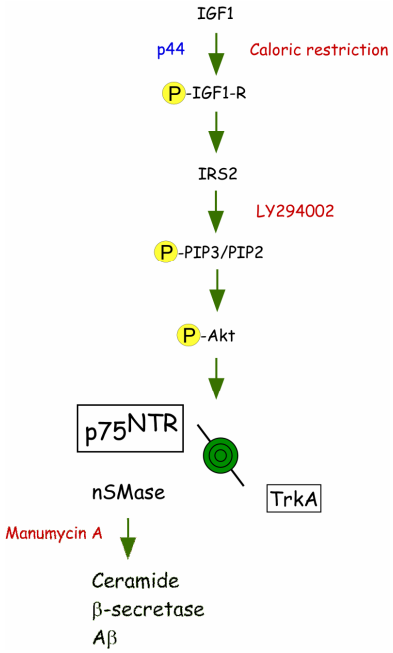
Two possible mechanisms might be responsible for p53 activation: p38 and JNK might activate directly p53 by stabilization of the protein and induced degradation of the inhibitory protein mdm-2. Alternatively, p38 and JNK might increase p53 transcription through activation of NF-kB.
P53 activation might then result in cell death through modulation of Bcl-2 family members, cytochrome C release and formation of the apoptosome.
Aβ can exist in different aggregation states. The initial formulation of the "amyloid cascade hypothesis" stated that the fibrillar form of Aβ was responsible for the neuronal death. The hypothesis was subsequently modified according to the observation that in human disease and in animal models of AD, amyloid plaques do not correlate in number, tempo and distribution with neurodegeneration and clinical dementia. Evidence is now accumulating that prefibrillar structures of Aβ, including activated monomers, soluble oligomers and protofibrils rather than the insoluble fibrils could be the active species ultimately responsible for the degenerative changes in neurons and the cognitive dysfunction in AD.
By using the same experimental model and different aggregation states of Aβ, we were able to show that: (a) soluble oligomers of Aβ exert a cytotoxic activity independent of p75NTR, (b) the expression of p75NTR exerts a protective role against the toxic activity of soluble oligomers, (c) this role is due to an active function of the juxtamembrane sequence of the cytoplasmic region of p75NTR and (d) the protective function is mediated by phosphatidylinositide 3-kinase (PI3K) activity (Costantini C. *et al.* Exp Cell Res. 2005 Nov 15;311(1):126-34).

2) Aging and Alzheimer's disease

Background:
Based on the age of onset, we can distinguish an early-onset Alzheimer's disease, that develops before 60 years and has been associated with mutations in PS and APP, and a late-onset Alzheimer's disease (>60), which accounts for the majority of AD cases (nearly 97%). Environmental and genetic

factors have been associated with LOAD, however aging is the single most important risk factor. In fact, the incidence of LOAD increases progressively with age without a plateau.
Since aging is the most important risk factor for sporadic AD, we looked for molecular pathway(s) that could explain this relationship. These molecules should be modulated in an age-dependent manner and should be able to increase Aβ generation as a consequence of this age-dependent modulation.
We focused on the lipid messenger ceramide because: 1) ceramide is regulated during aging: in fact the intracellular levels of ceramide increase progressively during aging in both cultured cells and the whole organ; 2) ceramide levels are altered in AD: in fact brains from AD patients contain approximately three times more ceramide when compared with age-matched controls. Moreover, it was shown that ceramide increases Aβ secretion and processing of APP at both α and β sites when added to cells in culture.

Results:
We were able to first demonstrate that neurotrophin receptors are differentially regulated in the brain during aging. In particular, p75NTR expression increases while TrkA expression decreases. As a consequence, neutral sphingomyelinase is activated and ceramide is produced, leading to an increase in β-secretase function and generation of amyloid-β peptides (Costantini C. *et al*. Biochem J. 2005 Oct 1;391(Pt 1):59-67). The latter event is currently considered the main pathogenetic event for the development of Alzheimer's disease. We then demonstrated that the differential regulation of p75NTR/TrkA is dependent on the activation of the IGF-1 pathway, known for its role in the regulation of longevity and age-associated diseases (Costantini C. *et al*. EMBO J. 2006 May 3;25(9):1997-2006). These results allowed us to explain in molecular terms the observation that aging is the single most important risk factor for Alzheimer's disease, according to the following picture:



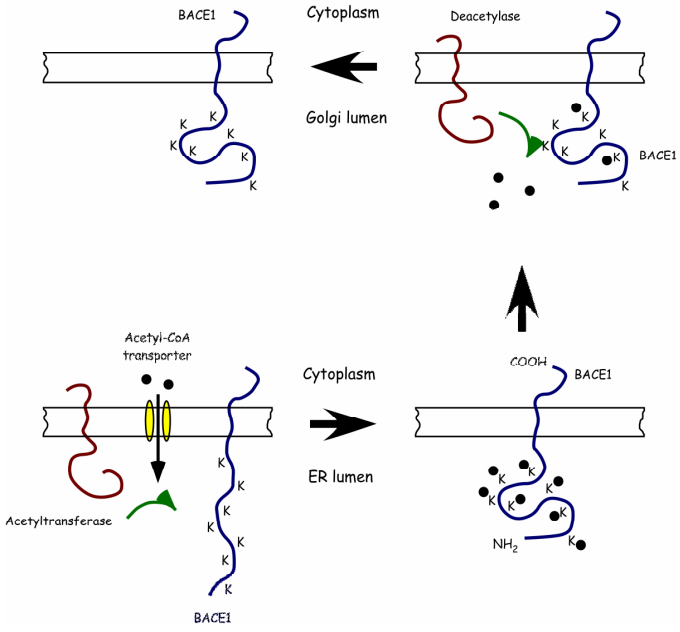
Moreover, the identification of this pathway revealed new possible targets for the prevention of Alzheimer's disease. For example, inhibitors of neutral sphingomyelinase were able to prevent the age-associated increase in ceramide levels and the subsequent accumulation of amyloid-β peptides. In addition, we were able to show that caloric restriction, that is a reduction in caloric intake with diet, previously known as the only intervention able to decrease the intrinsic rate of aging, interferes with the described pathway by blocking the activation of IGF-1 pathway in the brain. As a consequence,

caloric restriction prevents the accumulation of amyloid-β peptides during aging, thus indicating that a dietary regimen might represent a possible intervention to prevent Alzheimer's disease.

3) Therapeutic approaches to Alzheimer's disease: regulatory properties of a new acetylation system located in the secretory pathway

Background:
According to the "amyloid cascade hypothesis" novel therapeutic strategies that lower Aβ levelsor prevent the formation of the neurotoxic Aβ species are predicted to stop or slow down the progression of neurodegeneration in Alzheimer's disease.
BACE represents an ideal pharmacological target for different reasons: β-secretase cleavage of APP is the rate limiting step in the generation of amyloid-β peptides; β-secretase activity is not redundant; the phenotype of mice knock-out for BACE is apparently normal, without any evident physical, biochemical or behavioural defect.
BACE contains an N-terminal signal peptide that directs the nascent protein to the secretory pathway. In the endoplasmatic reticulum, the N-terminal signal peptide is removed and BACE1 is partially glycosilated, producing an immature form of about 60 kDa. BACE1 then moves to the different regions of the Golgi apparatus, where the prodomain is removed and BACE is further glycosilated, thus producing the final mature form with a molecular weight of about 70 kDa. BACE1 then to the plasmamembrane and it can recycle back to the trans-Golgi network through the endosomal compartment. BACE has a long half-life, so it recycles several times before being degraded in the lysosomes. Ceramide increases the steady-state levels of BACE with a post-translational mechanism, but the mechanisms is unknown.

Results:
Our results demonstrated that ceramide regulates BACE by modulating an acetylation system located in the secretory pathway. In particular, we were able to demonstrate for the first time that acetylation occurs in the lysine residues of several proteins, including BACE, in the lumen of the endoplasmic reticulum by an unknown acetyltransferase. The latter uses acetyl-CoA as a substrate and its transport in the lumen of the endoplasmic reticulum is mediated by an acetyl-CoA transporter located in the membrane of the vescicles. We were also able to demonstrate that the acetyl group is removed once the proteins are translocated in the Golgi apparatus by an unknown acetylase, thus indicating that acetylation is a transient modification occurring along the secretory pathway and probably involved in the regulation of the maturation of proteins, according to the following picture:



In fact, we were able to demonstrate that the stability and maturation of BACE and its translocation to the plasmamembrane are strongly modulated by the acetylation status (submitted for publication). Therefore, the interference with the acetylation system might represent an important pharmacological approach in order to prevent the increase in BACE levels during aging or its translocation to the cellular sites where amyloid peptides production occurs.

PUBLICATIONS

1. Della-Bianca V, Rossi F, Armato U, Dal-Pra I, Costantini C, Perini G, Politi V, Della Valle G. Neurotrophin p75 receptor is involved in neuronal damage by prion peptide-(106-126). J. Biol. Chem. 2001; 276: 38929-33.

2. Costantini C, Rossi F, Formaggio E, Bernardoni R, Cecconi D, Della-Bianca V. Characterization of the signaling pathway downstream p75 neurotrophin receptor involved in β-amyloid peptide-dependent cell death. J. Mol. Neurosci. 2005; 25: 141-156.

3. Costantini C, Kolasani RMK, Puglielli L. Ceramide and cholesterol: possible connections between normal aging of the brain and Alzheimer's disease. Just hypotheses or molecular pathways to be identified? Alzheimer's & Dementia 2005; 1: 43–50.

4. Costantini C, Weindruch R, Della Valle G, Puglielli L. A TrkA-to-p75NTR molecular switch activates amyloid β-peptide generation during aging. Biochem J 2005; 391: 59-67.

5. Costantini C, Della Bianca V, Formaggio E, Chiamulera C, Montresor A, Rossi F. The expression of p75 neurotrophin receptor protects against the neurotoxicity of soluble oligomers of β-amyloid. Exp. Cell. Res. 2005; 311: 126-134.

6. Costantini C, Scrable H, Puglielli L. An aging pathway controls the TrkA to p75NTR receptor switch and amyloid beta-peptide generation. EMBO J. 2006; 25:1997-2006.

COMMUNICATIONS

- **Society for Neuroscience** 34th Annual Meeting
October 23-27, 2004, San Diego, CA (USA).

Title: CALORIC RESTRICTION AND NEUTRAL SPHINGOMYELINASE INHIBITORS PREVENT THE AGE-ASSOCIATED ACTIVATION OF AMYLOID β-PEPTIDE GENERATION INDUCED BY THE p75NTR-CERAMIDE SIGNALING PATHWAY
C. Costantini, R. Weindruch, G. Della Valle, and L. Puglielli

- **Society for Neuroscience** 35th Annual Meeting
November 12-16, 2005, Washington, D.C. (USA)
Title: IGF-R, THE COMMON REGULATOR OF LIFE SPAN, ACTS UPSTREAM OF P75NTR IN THE REGULATION OF Aβ GENERATION DURING AGING
L.Puglielli, C.Costantini, and H.Scrable

Title: P44 TRANSGENIC MICE HAVE AN EARLY AND DRAMATIC INCREASE IN Aβ LEVELS IN THE BRAIN: A POSSIBLE ROLE OF IGF-1 SIGNALING IN THE REGULATION OF Aβ PRODUCTION DURING AGING
C.Costantini, H.Scrable, L.Puglielli

LECTURES

Alzheimer's and Neurodegenerative Diseases Seminar Series, November 2nd 2004, Madison, WI, USA
Title: The TrkA/p75NTR receptor system: a molecular link between aging and Alzheimer's disease.

Alzheimer's and Neurodegenerative Diseases Seminar Series, December 6th 2005, Madison, WI, USA
Title: Aging as a risk factor for Alzheimer's disease: in search for a molecular explanation.

Invited lecture, June 30th 2006, Bologna, Italy
Title: L'invecchiamento come fattore di rischio per la malattia di Alzheimer: una spiegazione molecolare

COURSES

Summer School "Microarray Technology and Bioinformatics", Camerino (Italy) 28 Agosto - 1 Settembre 2006