Tullio Florio

Full professor

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+39 010 353 8806

Education and training

1994
PhD in Neuropsychopharmacology and Toxicology
IDENTIFICATION OF A NOVEL TRANSDUCTION PATHWAY ACTIVATED BY SOMATOSTATIN TUROSIN PHOSOTATASE ACTIVITY - PhD TITLE
Università Federico II di Napoli - Napoli - IT

1990
Endocrinology specialist
Il-1beta effects on transdution mechanisms and prolactin release in lactotrophs - 50/50 e lode
Università Federico II di Napoli - Napoli - IT

1987
Medical Doctor degree
Pituitary adenylyl cyclase as intracellular mechanism mediating GHRH and somatostatin effects - 110/110 e lode
Università Federico II di Napoli - Napoli - IT

Academic experience

2002 - ONGOING
Associate Professor of Pharmacology
University of Genova - Genova - IT
Research and teaching

1998 - 2002
Associate Professor of Pharmacology
Università G. d'Annunzio di Chieti Pescara - Chieti - IT
Research and teaching

1997
Visiting Scientist
Vollum Institute - Oregon Health Sciences University - Portland (OR) - US
Research

1995 - 1996
Post-doctoral fellow
Università di Genova - Genova - IT

Research

1990 - 1994

Post-doctoral fellow

Vollum institute - Oregon Health Sciences University - Portland (OR) - US
Research

1988 - 1990

AIRC fellow

Università Federico II di Napoli - Napoli - IT
Research

Work experience

1996

Dirigente medico I livello (supplente)

Ospedale Policlinico San Martino - Genova - IT
Translational research

Language skills

Italian
Mother tongue

English
Proficient

Postgraduate research and teaching activity

Supervision of PhD students, residents and post-doctoral fellows

Supervisor of the following phD students:
Neurophysiology and Neuropharmacology Program:
Alessandro Corsaro (2003)
Alessandro Massa (2005)
Valentina Villa (2005)
Carola Porcile (2005)
Paolo Brambilla (2005)
Emanuela Repetto (2006)
Neuroscience Program:
Monica Gatti (2010)
Roberto Würth (2013)
Denise Galante (2016)
Agnese Solari (2018)
PI of the following Post-Docs:
Stefano Thellung
Mario Nizzari
Alessandro Corsaro
Monica Gatti
Roberto Wurth

Valentina Villa
Carola Porcile
Alessandro Massa
...

PhD committees membership

Faculty member of the PhD program In Neuroscience.
Coordinator of the 'Clinical and experimental neuroscience' branch in the Neurosicence program

Research interests

1) G PROTEIN MODULATION OF PHOSPHOTYROSINE PHOSPHATASE AS ANTIPROLIFERATIVE
MECHANISM IN TUMORS
Working as post-doc in P. Stork lab (Vollum Inst. Portland, USA), he contributed to the identification of a completely novel intracellular pathway by which somatostatin and dopamine G protein-coupled receptors exert direct antiproliferative effects in pancreas carcinoma cells: the activation of phosphotyrosine phosphatases (PTP). These studies, published in Science, demonstrated, for the first time, that PTP activity may be regulated by G proteins and that when active may induce a direct dephosphorylation of tyrosine kinase receptors (i.e. EGFR) to inhibit their activity (Pan M.-G., Florio T., Stork P.J.S. G-protein activation of a hormone-stimulated phosphatase in human tumor cells. Science 256: 1215-1217, 1992; Florio T., et al. Dopaminergic inhibition of DNA synthesis in pituitary tumor cells is associated with phosphotyrosine phosphatase activity. J Biol Chem 267: 24169-24172, 1992). This is nowadays considered a general pathway by which GPCR exert antiproliferative activity. In following studies, he identified that, in primary cultures of human GBM cells, somatostatin antiproliferative activity requires the activation of the receptor-like PTPη causing dephosphorylation of ERK1/2 (Massa A., et al The expression of the phosphotyrosine phosphatase DEP-1/PTPh dictates the responsivity of glioma cells to somatostatin inhibition of cell proliferation. J Biol Chem 279: 29004-29012, 2004).
2) CXCL12/CXCR4-CXCR7 CHEMOKINE AXIS AS AUTOCRINE PATHWAY SUSTAINING CANCER STEM CELL PROLIFERATION AND SELF-RENEWAL
This activity followed a relevant research area analysing the role of this chemokine as autocrine/paracrine factor that sustains tumor cell proliferation. He published several publications covering the activity of this system in human different tumors (breast and ovary carcinomas, glioblastoma, meningioma, pituitary adenoma). In particular, a CXCR4-dependent, src-mediated transactivation of EGFR was identified (Pattarozzi A., et al. 17b-estradiol promotes breast cancer cell proliferation inducing stromal cell-derived factor-1-mediated epidermal growth factor receptor transactivation: reversal by gefitinib pretreatment. Mol Pharmacol 73: 191-202, 2008), highlighting CXCR4 inhibition as a valuable target for antitumoral treatments. This assumption was further supported by the demonstration that the autocrine CXCR4 activation by CXCL12 plays a

relevant role in glioblastoma CSC functioning, causing the inhibition of the self-renewal activity of these cells (Gatti M., et al. Inhibition of CXCL12/CXCR4 autocrine/paracrine loop reduces viability of human glioblastoma stem-like cells affecting self-renewal activity Toxicology 314: 209-220, 2013).
3) IDENTIFICATION OF CSC-SELECTIVE MOLECULAR TARGETS MEDIATING ANTIPROLIFERATIVE
ACTIVITY IN CANCER STEM CELLS FROM HUMAN GBMs
Recent studies led to the identification of the intracellular chloride channel CLIC1 as regulatory
mechanism of CSC proliferation in GBM favouring CSC entering in the S phase. Importantly, although this channel is expressed also in normal stem cells, its activity is extremely transient in normal cells as compared to CSCs. Importantly he demonstrated that CLIC1 activity is selectively inhibited by metformin, representing the mechanism by which this drug is effective only in CSC, while is non-toxic for normal stem cells (Gritti M., et al. Metformin repositioning as antitumoral agent: selective antiproliferative effects in human glioblastoma stem cells, via inhibition of CLIC1-mediated ion current. Oncotarget 5: 11136-11152, 2014). Finally, as basis for the current project, the role PrPC as determinant of GBM CSC stemness and tumorigenicity was reported (Corsaro et al Oncotarget 7:38638, 2015).
4) MECHANISMS OF NEUROTOXICITY INDUCED BY MISFOLDED PETIDES (Abeta, PrP)
The intracellular mechanisms activated by prion protein fragments to cause astrocyte and microglia activation (release of cytokine or NO) and neuronal apoptosis were also characterized. In particular the modulation of calcium homeostasis and the activation of the MAP kinase p38 were identified as main effctors of the prion toxicity independently from amyloid formation. To this aim, more recently, we developed a novel experimental approach to study the relationship between the three-dimensional structure and the toxicity of PrP fragments. In particular the protease resistant core or PrP (corresponding to the amino acids 90-231) has been synthesized as recombinant protein. This protein can be refolded in a conformer that strucutrally resembles PrPC (high content of alpha helices, solubility, lack of toxicity) or (after controlled thermal denaturatuion) in a beta-rich isoform assuming features of the pathogenic PrPSc (high hydrophobicity, amyloid aggregation, neuronal toxicity). By using this model a relationship between the folding of the PrP fragment and its pro-apoptotic and gliotrophic activity was shown. More recently usnig fluorescein tags the mechanisms of internalization and the subecellular localization of this protein have been started to be analyzed in relationship with the toxic effects of the protein.

Grants

1999 - 2002

Molecular mechanisms induced by neurotoxic prion protein fragments physico-chemical and biological

characterization

Telethon - IT

Eur 123.949 - Pricipal investigator

All prion diseases recognize the prion protein (PrP) as etiologic agent. Alterations in the spatial structure of PrP originate a pathogenic form (PrPSc) that may be transmitted through infective, sporadic or genetic (due to point mutations in the PrP gene) modalities, causing neuronal death, glial proliferation and amyloid deposition. In this study we will evaluate the physico-chemical and neurotoxic properties of a recombinant PrP fragment corresponding to the protease resistant core of PrP (PrP 89-231 miniprion rMP). The physical characteristics of the rMP will be studied using circular dichroism, to determine the composition in a-helix and b-sheet structure, and fluorescence spectrometry to analyze its capability to aggregate in amyloid fibrils. The intracellular mechanisms involved in the neurotoxic effects of rMP will be studied comparing its effects with those of the peptide PrP106-126, that is known to retain some pathogenic features of PrPSc, but due to its limited size, cannot mimic all its characteristics. Using rat neuron cultures, we will characterize the neurodegenerative activity of rMP in comparison with PrP106-126, the induction of apoptosis and the intracellular pathways mediating these effects. We will evaluate the effects of rMP on the [Ca++]i, by means of microfluorimetric and electrophysiologic techniques. Then we will analyze the capability of the rMP to regulate the reactive oxygen species (ROS) production and the transcriptional activity of NFkB, that represent mediators of the neurodegeneration. Finally, we will study the effects of rMP on the activity of MAP kinases (ERK1/2, JNK, p38), that have been reported to be involved in the control of survival/apoptosis processes. Thus, we will characterize the neurodegenerative pathways activated by rMP, in correlation with its spatial conformation, allowing a better understanding of the neurotoxic mechanisms of PrPSc and foster the development of new therapies for these diseases.

2000 - 2001

Meccanismi intracellulari responsabili della risposta astrocitaria a stimoli neurodegenerativi ad opera delle MAP chinasi

MIUR (PRIN 1999) - IT

Eur 40.284 - Pricipal investigator

2002 - 2003

Meccanismi intracellulari responsabili della neurotossicità delle proteine prioniche studio degli effetti del PrP90-231

MIUR (PRIN 2001) - IT

Eur 59.909 - Pricipal investigator

2003 - 2004

Alutazione dell'attività neuroprotettiva del gabapentin nell neurotossicità da agenti antiblastici

PFIZER - IT

Eur 37.500 - Pricipal investigator

2003 - 2006

Sviluppo di molecole innovative in grado di curare malattie neurodegenerative e neuroinfiammatorie

MIUR (FIRB 2001) - IT

Eur 98.600 - Pricipal investigator

2004

Molecular mechanisms involved in SDF1 regulation of glioma proliferation and invasion

AIRC - IT

Eur 40.000 - Pricipal investigator

The overall goal of the project is the characterization of the role of the chemokine SDF1a and its receptor CXCR4, in the proliferation and invasion of glioma cells and the characterization of the intracellular mechanisms involved. Gliomas are devastating brain tumors, showing up to 100% of recurrences also after aggressive therapies. Recently a role for chemokines in the generation, progression and dissemination of many tumoral histotypes, including brain tumors, has been identified. A large number of chemokines and their receptors have been identified in tumoral cells and we previously described a direct role for the chemokine SDF1a on the proliferation of glioma cell lines. A further demonstration of the possible role of SDF1a in tumorigenesis derive from the observation that this chemokine can act as angiogenic factor. However the exact intracellular mechanisms mediating the proliferative and invasive mechanisms of SDF1a have not completely elucidated. In particular, the role of SDF1a in glioblastoma cells overexpressing mutated and constitutively active EGFR showing an aggressive behavior and reduced prognosis, has never been addressed. The identification of specific pathways and mechanisms activated in gliomas by novel molecules with proliferative, invasive and angiogenic properties, can be very relevant in consideration of the novel concept in tumor treatment proposing a targeted therapy based on the inhibition of intracellular pathways specific for tumoral cells. Moreover, we will test the possibility of cell therapy using neural stem cells to affect the proliferation of the tumoral cells in vivo and in vitro glioma models, via both a direct effect due to the wide range of products they release and after transduction with a SDF1 antagonist (mutation P2G in the SDF1 sequence)

The specific aims of the project will be the following:

- **To characterize the expression of the CXC chemokines and their receptors in brain tumors.**
- **To identify the role of the chemokine SDF1a in the proliferation of primary cultures of human gliomas, in comparison with human glioblastoma cell lines.** .
- **To identify the role of the cross-talk between SDF1/CXCR4 and the EGFR as intracellular mechanism involved in the proliferative effects of SDF1a.**

- **To evaluate the role of the activation of NFkB in the proliferative activity of SDF1.**
- **To evaluate the effects of SDF1a on the invasive characteristics of glioma cells testing their migration and the possible intracellular mechani**

2004 - 2006

Studio su farmaci antiproliferativi per adenomi ipofisari umani clinicamente non funzionanti

IPSEN-BEAUFUR / BIOMEASURE Inc. - FR

Eur 80.000 - Pricipal investigator

2005

Ruolo dello ZD1839 sulla proliferazione di cellule di carcinoma mammario ER+ e ER-

ASTRA-ZENECA - IT

Eur 25.000 - Pricipal investigator

2005

Applicazione di tecnologie innovative per lo studio molecolare e cellulare in modelli di Parkinson e altre m. neurodegenerative

CNR-MIUR (FISR) - IT

Eur 48.162 - Pricipal investigator

2006 - 2008

Demenza di Alzheimer studio dell'effetto di mutazioni del gene PS1 sul signalling e nell'induzione della morte neuronale

Fondazione CARIGE - IT

Eur 165.000 - Pricipal investigator

2007 - 2008

Il PrP90-231 come modello per lo studio dei rapporti struttura-attività neurotossica e gliotrofica della proteina prionica

MIUR (PRIN 2006) - IT

Eur 57.714 - Pricipal investigator

2008

Evaluation of dopastatin effects on non functioning human pituitary adenomas a pharmacogenomic approach

IPSEN-BEAUFUR / BIOMEASURE Inc. - FR

Eur 50.000 - Pricipal investigator

2008 - 2009

Biologia delle cellule staminali di carcinoma mammario felino come modello di studio del carcinoma umano

MISAN (Ricerca Corrente IZS-PLV) - IT
Eur 40.480 - Pricipal investigator

2008 - 2010

Caratterizzazione biochimica e cellulare di un'isoforma tossica della proteina prionica ricombinante

Compagnia di San Paolo - IT
Eur 190.000 - Pricipal investigator

- Il progetto si propone di individuare, mediante l'uso di un modello sperimentale originale e innovativo, possibili approcci farmacologici per il trattamento delle malattie prioniche. Intendiamo infatti sviluppare un modello sperimentale innovativo che consente di valutare l'efficacia di composti sia in grado di interferire con le vie di morte attivate dalla proteina prionica patogena, sia di composti in grado di prevenire, bloccare o revertire la conformazione strutturale di questa proteina responsabile della degenerazione neuronale. Infatti il nostro modello, a differenza di precedenti studi effettuati su piccoli peptidi sintetici derivati dalla proteina prionica, utilizzerà una proteina prionica ricombinante che mediante specifici protocolli sperimentali potrà assumere sia la configurazione non patogena (cellulare) normalmente presente nel sistema nervoso centrale umano, sia la configurazione neurotossica che si sviluppa in corso di encefalopatie prioniche. Destinatari potenziali dell'iniziativa saranno quindi soggetti affetti da tali patologie. Gli obbiettivi fondamentali di questa iniziativa sono l'aumento delle conoscenze riguardo la patogenesi delle malattie prioniche e lo sviluppo di nuovi approcci farmacologici. A tale scopo la nostra priorità sarà l'identificazione degli eventi intracellulari coinvolti nella trasduzione dell'attività biologica del hPrP90-231 (morte neuronale e proliferazione gliale). L'identificazione di tali meccanismi potrà favorire anche lo sviluppo di molecole in grado di bloccare questo processo e che quindi rappresentano potenziali nuovi approcci terapeutici. Tali obbiettivi sono in pieno accordo con le finalità istituzionali di un Dipartimento universitario.

2010 - 2011

Meccanismi di tossicità del frammento 89-230 della PrP murina conmutazioni patogene antagonismo con acridine innovative

MIUR (PRIN 2008) - IT
Eur 52.413 - Pricipal investigator

2010 - 2011

Analisi delle alterazioni genetiche ed epigenetiche nelle neoplasie cerebrali e loro significato prognostico

Regione Liguria (Bando Regionale Ricerca Sanitaria 2009) - IT

Eur 36.600 - Pricipal investigator

2010 - 2011

Biologia delle cellule staminali di carcinoma mammario felino come modello di studio del carcinoma umano

Fondazione CARIGE - IT

Eur 50.000 - Pricipal investigator

2010 - 2012

Role of CXCR4 and CXCR7 in glioma-derived stem cell proliferation migration and invasiveness

AIRC - IT

Eur 150.000 - Pricipal investigator

Chemokines (CKs) and their receptors, known as critical mediators of cell proliferation, migration and homing during immune surveillance, are also involved in cancer cell survival, proliferation, invasion and tumoral angiogenesis. In particular, CK receptors CXCR4 and CXCR7 and their ligands, SDF1/CXCL12 and I-TAC/CXCL11, are frequently expressed by human cancer cells and tumor neovessels. However, the exact mechanisms by which CXCR4 and CXCR7 enhance tumor growth and their involvement in disease progression are still unknown. Malignant tumors, including glioblastoma multiforme (GBM), contain cancer stem cells or tumor initiating cells (TICs), which constitute a cell subpopulation with the capacity of self-renewing, multipotent differentiation, tumorigenicity and drug resistance. It was reported that, in virtue of these characteristics, TICs are able to overtake the current treatments and are responsible for tumor recurrence and spreading. GBM is the most common glial neoplasm characterized by aggressive biological behavior, diffuse infiltrative growth, and resistance to chemo- and radiotherapy.

It was proposed that CK receptor signaling may act onTICs inducing cell proliferation, survival and migration. The present project reports on the use of cellular, molecular and pharmacological approaches to extensively characterize the role of CXCR4 and CXCR7 in processes leading to GBM growth using human GBM TIC cultures as experimental model.

TICs, isolated from 7 surgical specimens and expanded in vitro, have been already characterized for cancer stem cell marker expression and tumorigenicity in vivo. GBM TICs

grow in vitro as neurospheres and maintain an undifferentiated state, as indicated by morphology and expression of stem cell markers such as CD133 and nestin, are capable to self-renew, initiate xenograft tumors and differentiate into cells expressing glial and neuronal markers. Altogether, these features indicate that these TIC cultures are a reliable in vitro and in vivo model for studying GBM development and drug sensitivity.

The potential impact of the proposed research is the identification of the CK system as possible new therapeutic target to block TIC tumorigenicity

and invasiveness. In this perspective the combination of conventional drugs with inhibitors of CXCR4 and CXCR7 may result in a powerful inhibition of TICs growth to obtain a more efficacious treatment of GBM.

2012 - 2016

Patologie neurodegenerative e danno cerebrale meccanismi cellulari e molecolari alla base del deterioramento cognitivo

MIUR (Accordi di Programma FIRB 2011) - IT

Eur 643.469 - Pricipal investigator

2013 - 2014

Identificazione dei meccanismi di resistenza e nuovi target molecolari in cellule staminali derivate da mesotelioma pleurico

Fondazione Buzzi Unicem ONLUS - IT

Eur 50.000 - Pricipal investigator

2013 - 2015

Cancer stem cell transdifferentiation in glioblastoma angiogenesis and invasiveness role of CXCR4 and CXCR7

AIRC - IT

Eur 240.000 - Pricipal investigator

2014 - 2016

Structural insights on oligomers of A peptides in the presence of PrPC

Alzheimer's Association - US

USD 20.000 - Pricipal investigator

2014 - 2017

Studio dei dettagli molecolari della conversione della PrP nella forma neurotossica per individuare nuovi target farmacologici

Compagnia di San Paolo - IT

Eur 360.000 - Pricipal investigator

2016 - 2018

Ruolo di protein fosfatasi nel cancro della mammella possibili target farmacologici e biomarcatori predittivi di risposta e resistenza al trastuzumab

Compagnia di San Paolo - IT

Eur. 180.000 - Pricipal investigator

2018 - ONGOING

Study on molecular players controlling glioblastoma stem

cell reprogramming in non-tumorigenic cells as pharmacological strategy
Fondazione Giovanni Celeghin - IT
Eur 170.500 - Pricipal investigator

Editorial activity

Contribution to the writing of the **II editon** of the textbook "Cardio-Angio Farmacologia" di A. Marino, Piccin Editore (Padova) (1994)
Contribution to the writing of the **italian editon** of the textbook "Farmacologia Medica" di Brody, Lerner, Minneman, Neu, Edizioni EdiSES (Napoli) (1997)
Contribution to the writing of the textbook "Farmacologia. Principi di base ed applicazioni terapeutiche" di F. Rossi, V. Cuomo, C. Riccardi, Edizioni Minerva Medica (Torino) (2005)
Contribution to the writing of the **II editon** of the textbook "Farmacologia. Principi di base ed applicazioni terapeutiche" di F. Rossi, V. Cuomo, C. Riccardi, Edizioni Minerva Medica (Torino) (2011)
Contribution to the writing of the textbook "Biotecnologie" di Aldo Pagano, Helen Kreuzer, Adrianne Massey, Zanichelli Editore (Bologna) (2014)
Contribution to the writing of the **III editon** of the textbook "Farmacologia. Principi di base ed applicazioni terapeutiche" di F. Rossi, V. Cuomo, C. Riccardi, Edizioni Minerva Medica (Torino) (2016)
Contribution to the writing of the **IV editon** of the textbook "Farmacologia. Principi di base ed applicazioni terapeutiche" di F. Rossi, V. Cuomo, C. Riccardi, Edizioni Minerva Medica (Torino) (2018)
Editorial Board member for the following scientific journals:
PHARMACOLOGICAL RESEARCH (2009-2011)
ANTI-CANCER DRUGS (dal 1/1/2009 to date)
WORLD JOURNAL OF PHARMACOLOGY (dal 1/1/2012 to date)
THE SCIENTIFIC WORLD JOURNAL (dal 1/1/2012 to date) (Manuscript Managing Editor)
INTERNATIONAL JOURNAL OF COMPARATIVE ONCOLOGY (2012-2015)
JOURNAL OF HORMONES (2013-2016) (Manuscript Managing Editor)
FRONTIERS IN NEUROSCIENCE (sect. Neuroendocrine Science) (Review Editor) (dal 1/8/2015 to date)
FRONTIERS IN ENDOCRINOLOGY (sect. Neuroendocrine Science) (Review Editor) (dal 1/8/2015 to date)
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES (Editorial Board, sect. 'Molecular Pathology, Diagnostics, and Therapeutics (dal 1/10/2018 to date)
Reviewer for the following NATIONAL grants:

- *CIVR (MIUR) 2005*
- *ANVUR - VQR 2004-2010 2012*
- *COFIN-PRIN 2005/2007/2009 (MIUR) 2006, 2008, 2011*
- *Programma Rientro dei Cervelli (MIUR) 2008*
- *Programma "Futuro in ricerca" (MIUR-FIRB) 2009, 2012, 2013*
- *Fondazione Dal Monte (Bologna) 2008-2017*
- *Progetti Ateneo Università La Sapienza (Roma) 2014*

- *Revisore ex-post Progetti FIRB (MIUR) 2017*
- *Bando Roche per la Ricerca 2018*

Reviewer for the following INTERNATIONAL grants:
Austria

- *Wittgenstein Award (Austrian Federal Ministry for Science and Research) 2008*

Belgium

- *Research Foundation - Flanders - FWO (Fonds Wetenschappelijk Onderzoek-Vlaanderen)*
  2013, 2015, 2016, 2018
- *Research Council of KU Leuven 2016*

EU

- *European Science Foundation (Exploratory Workshop Scheme) 2012*

France

- *Fédération pour la Recherche sur le Cerveau (FRC) 2009*
- *Agence Nationale de la Recerche (ANR) 2012, 2015*
- *CNRS & INSERM ATIP-AVENIR Program 2016*

UK

- *The Wellcome Trust "Research Training Fellowship" 2013*
- *Pancreatic Cancer Research Fund 2018*

Hong Kong

- *Research Grants Council 2010*

India

- *Biomedical Resaerch Fellowship Pogramme (India Alliance System) 2011, 2018*

Ireland

- *Health Research Board (HRB) (Ireland Support to Health Research) 2011*

New Zaeland

- *Cancer Society of New Zealand 2017*

Poland

- *National Science Centre 2018*

Czech Republic

- *Czech Science Foundation 2014, 2015*

Switzerkand

- *Swiss Cancer League 2015*

REVIEWER OF SCIENTIFIC ARTICLES FOR 140 JOURNALS INCLUDING: Nature Reviews Neuroscience, Nature Communications, Journal of Neuroscience, Endocrinology, Journa