SCUOLA NORMALE SUPERIORE

RELAZIONE ATTIVITA' ANNUALE DEI PERFEZIONANDI/DOTTORANDI – TERZO/QUARTO ANNO
REPORT ON THE PHD ACTIVITY – THIRD/FORTH YEAR

NAME AND SURNAME	Federica Anastasi
PHD COURSE	Nanoscienze III anno

CORSI FREQUENTATI CON SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (WITH FINAL EXAM)	**VOTAZIONE RIPORTATA MARK**	**NUMERO DI ORE HOURS**
NS0638- **Biophysical Sciences** - Ciclo di seminari (esame sostenuto pochi giorni dopo il passaggio di anno II -> III)	idoneo	40

CORSI FREQUENTATI SENZA SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (ATTENDANCE ONLY)	**NUMERO DI ORE HOURS**

ALTRE ATTIVITÀ FORMATIVE (SEMINARI, WORKSHOP, SCUOLE ESTIVE, ECC.) – DESCRIZIONE OTHER PHD ORIENTED ACTIVITIES (SEMINARS, WORKSHOPS, SUMMER SCHOOLS, ETC) – DESCRIPTION	**NUMERO DI ORE HOURS**
Seminar - Melissa Santi – ''3D cell models and personalized cancer nanomedicine''	1
Seminar - Francesco Cardarelli – ''Capturing dynamic molecular processes in a trafficking organelle: the lysosome case''	1
Seminar - Maarten Altelaar (Utrecht University) – ''MS-based proteomics profiling of kinome-wide activation states and extracellular vesicles'' – CFF 2019 UNIPI	1
Seminar - Ranieri Bizzarri – ''Single Cell Optical imaging: the role of spatial hierarchy for life at work''	1
Seminar - Angeliki Louvi (Yale School of medicine) – ''Investigating Brain Disease: from Patients to Model Organisms and Beyond''	1
Seminar - Dott. Andrea Berti (Reparto Investigazioni Scientifiche Carabinieri, Roma) – ''Present and future of Forensic Biology"	1
Seminar – Giulio Caracciolo – ''The King and the Crown: Impact of the Nanoparticle-Protein Corona in Nanomedicine''	1
Colloqui Classe di Scienze - Arthur Mallay Lesk – "The architecture of proteins''	1
Seminar – Matteo Agostini – ''Microfluidics and biosensing: two fields that can meet with surface acoustic waves''	1
Seminar – Ennio Tasciotti (Houston Methodist Research Institute) – ''Biomimetic materials: opportunities in biomedicine''	1
Cycle of seminar: PhD students and researchers Fondazione Pisana per la Scienza	15

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ATTIVITÀ DI RICERCA SVOLTA (MAX. 8.000 CARATTERI)*
RESEARCH ACTIVITY (MAX. 8000 CHARACTERS)

Project introduction

EVs released from cancer cells contain tumor derived material, many studies have focused on their role in cancer as mediators of cross-talk between cancer cells and the immune system and as a potential source of biomarkers. Longitudinal analysis of individual animals enables to better resolve molecular changes due to disease progression or therapeutic intervention, because it circumvents the high variance that can exist between different individuals. The maximum volume of serum that can be drawn from an adult mouse for longitudinal studies is approximately 75 µl every 14 days. To date no methods have been reported that are able to analyze the proteome of small extracellular vesicles (*s*EVs) from such low serum amounts.

Research activity

I have previously developed an ultrasensitive workflow for the analysis of the exosome proteome and demonstrated that size exclusion chromatography (SEC) performs is superior, in terms of specificity, to established precipitation-based methods for purifying *s*EVs from serum. During this year I have further optimized and scaled-down the workflow for the isolation and proteomics analysis of *s*EVs from low volumes of serum thereby enabling the longitudinal analysis of individual mice:

- **Down-scaling** of the procedure to 50 µl of serum: initial tests resulted in the extraction of 2.6±0.26 µg of protein, which led to identification of 181±60 protein groups.
- **Protein digestion optimization:** a possible cause of the high variation in the number of identified proteins is an incomplete digestion: the percentage of zero missed-cleavage peptides was just 63% (significantly lower than the 90% threshold by which we QC our measurements). Since incomplete proteolytic digestion leads to a decreased number of protein identification, poor reproducibility and poor quantitation precision, I sought to further optimize the digestion. Two different digestion conditions were tested and optimized. The optimized digestion reduced variability and increased the number of protein identifications, with 277±2 protein groups identified from the *s*EVs isolated from 50 µl normal mouse serum.
- **Comparison between serum and plasma as starting sample:** It is well established that during the preparation of serum the fibrin clot incorporates a large number of blood proteins, and which may also affect the number of EVs that can be isolated from serum. To evaluate the differences between serum and plasma, the procedure was applied on 3 serum and 3 plasma samples (50 µl). The number of identified proteins was much more variable for the plasma derived *s*EVs, with 213±4 protein groups identified from serum-*s*EVs and 206±88 from plasma-*s*EVs. Three proteins are mostly responsible for the differences between plasma and serum sEVs samples: all part of the fibrinogen family (FGa, Fgb, Fgg), and indicate that the SEC did not isolate *s*EVs from the fibrinogen proteins.

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- **Workflow applicability on glioblastoma multiforme (GBM) sEVs:** The SEC-EV procedure and microproteomics method was then used to compare the proteins from *s*EVs of control and GBM inoculated mice (n=6), using just 50 µl of serum from each mouse. 6 protein groups were detected at higher levels in the *s*EVs obtained from the GBM mice, and 7 proteins detected at lower levels. Some of the specific proteins that were found upregulated in the GBM-*s*EVs (Mhy9, Tln-1, Col1a1) are known mediators of tumor invasion and associated with poor prognosis. Myosin IIA (Myh9) is involved in cell migration, tumor invasion and metastasis, Talin1 (Tln-1) plays an important role in the formation of focal adhesion and motility of glioma cells. Col1a1 in two glioma stem cell lines demonstrated its role in promoting invasion and neurosphere-initiating capacity. Interestingly, the protein reelin (Reln) was detected at higher levels in the GBM-*s*EVs; Reln is an extracellular matrix serine protease that plays a crucial role in the layering of neurons and neuronal migration. Reln regulates cell proliferation through the activation of the PI3K/AKT pathway and has been reported to promote myeloma progression.
- **Longitudinal study of 4 GBM mouse model:** The workflow has been applied on the sEVs isolated from 50 µl serum of 4 glioblastoma mice models. Serum was withdrawn 15 days before tumor inoculation, 12 days after the tumor inoculation (T1, presymptomatic phase) and 21 days after the tumor inoculation (T2, symptomatic phase). Since longitudinal samples must contend with the temporal evolution of the data, and different animals may have different initial conditions, a linear mixed effect model was used to analyze the data. 8 proteins were found to exhibit a statistically significant effect, most of which increased with tumor development: angiopoietin-1, laminin subunit 1, serglycin, vitronectin and myosin 9, all proteins related with tumor progression and metastatic potential.
  (Manuscript in preparation)

Other collaborations:

- Neuro exosome characterization – Application of the newly developed procedure to brain exosomes purified from the plasma of Parkinson's disease, Alzheimer's disease and SLA.
- Glioblastoma extracellular vesicle membrane-protein characterization – sEVs have been isolated from primary cell cultures of glioblastoma patients.

Future activities:

Accepted oral communication: Microproteomics workflow for sEVs biomarker discovery: enabling single mouse analysis on longitudinal models'' – 6-8th November – Palermo (PA)
Visiting PhD student: from December 2019 to May 2020 in Utrecht (The Netherlands)

*se si intende sottoporre una relazione di ricerca più estesa, utilizzare il campo per una descrizione sintetica e allegare il documento in formato .pdf

If you are going to submit a longer report, please fill the box with a synthetic abstract and attach a document in pdf format

EVENTUALI PUBBLICAZIONI **PUBLICATIONS (IF AVAILABLE)**

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NOME DEL RELATORE THESIS ADVISOR
Liam McDonnell (Fondazione Pisana per la Scienza) Stefano Luin – Supervisore SNS

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