UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.5

Proponent: Prof. Ferdinando Chiaradonna

Project Title: Definition of the role of glycolysis-related processes in DNA damage response and cancer chemoresistance

Scientific background and 'open issues'

Maintaining genome integrity is a key biological process required to suppress diseases. In response to DNA damage, cells elicit a signalling cascade resulting in the activation of specific repair machinery. The DNA damage response (DDR) has been well-studied, extending our knowledge about DDR pathways. Thereby, it has emerged that cellular metabolism is tightly connected to DNA repair and DNA damage [1]. An extensive metabolic reprogramming and alterations in DNA repair pathways are present in cancer cells, emphasizing the links between metabolism and DDR. The increased use of anaerobic glycolysis (Warburg effect) and its sideways branches is one of the main metabolic changes during carcinogenesis. Of note, a part of glucose is redirected in Hexosamine Biosynthetic Pathway (HBP). This pathway provides the substrate for threonine and serine O-glycosylation (O-GlcNAc) of nuclear and cytoplasmic proteins. Importantly, protein phosphorylation and O-GlcNAc, on serine and threonine, may have additive or opposite effect on protein function. Some evidence indicates that increased flux through the HBP is necessary for pancreatic and breast cancer cell survival under DNA damage and chemoresistance as well [2,3]. On the other hands, methylglyoxal (MGO), a carcinogenic metabolite produced mainly as a side-product of glycolysis, especially in glycolytic cancer cells, generates DNA adducts and histones glycation, both affecting chromatin architecture, and able to induce or interfere with DNA damage and repair [4]. Both glycolysis-dependent effects on DNA-related processes are not much explored and several issues still need to be addressed. For instance, must be elucidated the function of the DNA repair proteins O-GlcNAc, almost all phosphorylated, to understand its function in protein activity. Second, must be well-addressed the role of MGO, since it has a dual function, oncogenic in primary tumor, by increasing genomic instability, oncosuppressor in advanced tumor, by inducing cell death. Third, how these two glycolytic-related processes are connected, also never has been addressed and this must be important in the DNA repair mechanisms. Finally, our published and preliminary results, suggest that both processes, may be good therapeutic targets either in primary or advanced tumor, providing a novel field of investigation that urges to be in-depth elucidated.

Objectives

- Identification of O-glycosylation protein status upon DNA damage response, with a particular focus on DDR proteins. The analysis will be performed in glycolytic and respirative cancer cell models to define their grade of dependency on HBP flux for DNA repair.
- Characterisation of the O-glycosylation role in identified DDR proteins by creation and expression in cancer cell models of mutated proteins in specific O-glycosylated amino acids.
- Analysis of the effect of these mutations in response to DNA damage agents and during the acquisition of chemoresistance.
- Effect of MGO accumulation on histone proteins and its effect on cancer cells ability to cope with DNA damage, in both glycolytic and respirative cancer cell models.
- Effect of the inhibition/activation of HBP as well as modulation of MGO levels, as single or combined analysis, in cancer cells ability to survive under DNA damage drugs and in chemoresistance.

Methodologies

For this project will be used these methodologies and experimental procedures:
Seahorse technology;
Click Chemistry Azide-Alkyne Cycloaddition;
Immunoprecipitation;
Western blot analysis;
LC-MS/MS analysis;
Generation of protein point mutation;
cDNA in vivo expression;
Cross-linking-based proteomic strategy (XL-MS)
Operetta technology;
2D-TAU gel analysis;
CRISPR/Cas9;

Collaboration

The project already plans a continuative collaboration with Prof. Domenica Scumaci, associate professor of Biochemistry at Department of Experimental and Clinical Medicine of the Magna Græcia University of Catanzaro. Prof. Scumaci has the necessary expertise to perform part of the project, in particular she is a recognized expert in proteomics analysis with a particular focus on identification of protein post translational modifications, histone and chromatin purification. The PhD student, in agreement with both tutor and supervisor, will spend a period in Scumaci's lab to learn and apply the proteomics principles.

Project's Sustainability & Mobility

Coherence

The Tumor Biochemistry lab, whose responsible is prof Ferdinando Chiaradonna, has all the competences to follow a project on cancer cell metabolism, from the metabolic characterization of cancer cells to DNA repair/damage analysis, to proteomics analysis.

Pertinent articles authored by the proponent

1. Ricciardiello F, Gang Y, Palorini R, Li Q, Giampà M, Zhao F, You L, La Ferla B, De Vitto H, Guan W, Gu J, Zhang T, Zhao Y, Chiaradonna F*. Hexosamine pathway inhibition overcomes pancreatic cancer resistance to gemcitabine through unfolded protein response and EGFR-Akt pathway modulation. Oncogene. 2020 May;39(20):4103-4117.
2. Ricciardiello F, Votta G, Palorini R, Raccagni I, Brunelli L, Paiotta A, Tinelli F, D'Orazio G, Valtorta S, De Gioia L, Pastorelli R, Moresco RM, La Ferla B, Chiaradonna F*. Inhibition of the Hexosamine Biosynthetic Pathway by targeting PGM3 causes breast cancer growth arrest and apoptosis. Cell Death Dis. 2018 Mar 7;9(3):377. *Corresponding Author

Possible foreign institution for mobility

Prof. Andrea Ventura, Cancer Biology and Genetics Program Memorial Sloan Kettering Cancer Center, New York, NY, USA

References

[1] Turgeon MO, Perry NJS, Poulogiannis G. DNA Damage, Repair, and Cancer Metabolism. Front. Oncol. 2018, 8, 15.
[2] Ricciardiello F, Gang Y, Palorini R, Li Q, Giampà M, Zhao F, You L, La Ferla B, De Vitto H, Guan W, Gu J, Zhang T, Zhao Y, **Chiaradonna F**. Hexosamine pathway inhibition overcomes pancreatic cancer resistance to gemcitabine through unfolded protein response and EGFR-Akt pathway modulation. Oncogene. 2020 39(20).
[3] Ricciardiello F, Votta G, Palorini R, Raccagni I, Brunelli L, Paiotta A, Tinelli F, D'Orazio G, Valtorta S, De Gioia L, Pastorelli R, Moresco RM, La Ferla B, **Chiaradonna F**. Inhibition of the Hexosamine Biosynthetic Pathway by targeting PGM3 causes breast cancer growth arrest and apoptosis. Cell Death Dis. 2018, 7;9(3).
[4] Epigenetic meets metabolism: novel vulnerabilities to fight cancer. **Scumaci D**, Zheng Q. Cell Commun Signal. 2023, 21;21(1).
[5] Rossi M, Altomare E, Botta C, Gallo Cantafio ME, Sarvide S, Caracciolo D, Riillo C, Gaspari M, Taverna D, Conforti F, Critelli P, Bertucci B, Iannone M, Polerà N, **Scumaci D**, Arbitrio M, Amodio N, Di Martino MT, Paiva B, Tagliaferri P, Tassone P.miR-21 antagonism abrogates Th17 tumor promoting functions in multiple myeloma. Leukemia 2021, 35(3).
[6] Liu Y, Chen Q, Zhang N, Zhang K, Dou T, Cao Y, Liu Y, Li K, Hao X, Xie X, Li W, Ren Y, Zhang J. Proteomic profiling and genome-wide mapping of O-GlcNAc chromatin-associated proteins reveal an O-GlcNAc-regulated genotoxic stress response. Nat Commun. 2020,11,5898.
[7] Klykov O, Steigenberger B, Pektaş S, Fasci D, Heck AJR, Scheltema RA. Efficient and robust proteome-wide approaches for cross-linking mass spectrometry. Nat Protoc. 2018 Dec;13(12).