Research Project Proposal
Academic year 2021-2022
Máster en Investigación Biomédica

Project Nº 34
Title: Epigenetic deregulation of endogenous retroviral genes in the origin of human cancers
Department of Hemato-Oncology, Center for Applied Medical Research (CIMA), University of Navarra
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Summary:

For millions of years, endogenous retroviral genes (ERVs) have remained transcriptionally silent within mammalian genomes by epigenetic mechanisms (Kassiotis and Stoye, 2016). Modern anticancer therapies targeting the epigenetic machinery awaken ERV expression, inducing antiviral responses that eliminate tumors through IFN-mediated immune signaling (Chiappinelli et al., 2015; Roulois et al., 2015). Our laboratory has recently found that ERVs are sensed by RIG-I and MDA5 helicases that act via ATP hydrolysis, which abruptly depletes ATP and activates compensatory respiration of mitochondria, thereby reversing the Warburg effect. However, hyperactive mitochondrial succinate dehydrogenase produced ROS and consequent caspase-independent tumor necroptosis that could be further promoted by BCL2 inhibitors, demonstrating synergy between epigenetic inhibitors and pro-apoptotic agents in multiple cancer types (Fresquet et al., 2021). Building on these results we here hypothesize that ERVs play a role in the origination of human cancers. More specifically we postulate, that genetic mutations in epigenetic enzymes that occur at earliest stages of tumorigenesis repress ERV expression, which limits IFN-driven immune responses and reduces basal energy consumption, collectively facilitating malignant transformation (Griffin et al., 2021; Wijewardhane et al., 2021; Yizhak et al., 2019). In this proposal we will evaluate this hypothesis in human and mouse cell line models from different hematological malignancies, which will be genetically manipulated by CRISPR-cas9 to reproduce common mutations in CREBBP, KMT2D, EZH2, DNMT3A, TET2 and ARID1A epigenetic regulators. In parallel, genetically modified mice carrying mutations in Crebbpfl/fl, Ezh2Y641F or Dnmt3aR878H, which progressively develop lymphoid and myeloid cancers, will be characterized. Mutation-induced transcriptional (RNAseq), genetic (WES) and epigenetic (ATACseq) variations genome-wide and on heterochromatic ERV gene loci will be defined in the tumor models. Changes in specific ERV gene/s or family genes will be functionally validated in in vitro cell line systems and in in vivo immunocompetent models of disease. We expect to provide comprehensive data on how ERV deregulation caused by early epigenetic gene mutations contributes to the progressive development of tumors at cell intrinsic and extrinsic levels, which may shed light on novel strategies to prevent and treat human cancer.

- **References**:

- Chiappinelli, K.B., et al. (2015). Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. Cell 162, 974-986.
- Fresquet, V., et al. (2021). Endogenous Retroelement Activation by Epigenetic Therapy Reverses the Warburg Effect and Elicits Mitochondrial-Mediated Cancer Cell Death. Cancer Discov 11, 1268-1285.
- Griffin, G.K., et al. (2021). Epigenetic silencing by SETDB1 suppresses tumour intrinsic immunogenicity. Nature. 10.1038/s41586-021-03520-4.
- Kassiotis, G., and Stoye, J.P. (2016). Immune responses to endogenous retroelements: taking the bad with the good. Nat Rev Immunol 16, 207-219.
- Roulois, D., et al. (2015). DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts. Cell 162, 961-973.
- Wijewardhane, N., Dressler, L., and Ciccarelli, F.D. (2021). Normal Somatic Mutations in Cancer

Transformation. Cancer Cell 39, 125-129.

- Yizhak, K., et al. (2019). RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. Science 364. 10.1126/science
- Chiappinelli, K.B., Strissel, P.L., Desrichard, A., Li, H., Henke, C., Akman, B., Hein, A., Rote, N.S., Cope, L.M., Snyder, A., et al. (2015). Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses. Cell 162, 974-986. 10.1016/j.cell.2015.07.011.
- Fresquet, V., Garcia-Barchino, M.J., Larrayoz, M., Celay, J., Vicente, C., Fernandez-Galilea, M., Larrayoz, M.J., Calasanz, M.J., Panizo, C., Junza, A., et al. (2021). Endogenous Retroelement Activation by Epigenetic Therapy Reverses the Warburg Effect and Elicits Mitochondrial-Mediated Cancer Cell Death. Cancer Discov 11, 1268-1285. 10.1158/2159-8290.CD-20-1065.
- Griffin, G.K., Wu, J., Iracheta-Vellve, A., Patti, J.C., Hsu, J., Davis, T., Dele-Oni, D., Du, P.P., Halawi, A.G., Ishizuka, J.J., et al. (2021). Epigenetic silencing by SETDB1 suppresses tumour intrinsic immunogenicity. Nature. 10.1038/s41586-021-03520-4.
- Kassiotis, G., and Stoye, J.P. (2016). Immune responses to endogenous retroelements: taking the bad with the good. Nat Rev Immunol 16, 207-219. 10.1038/nri.2016.27.
- Roulois, D., Loo Yau, H., Singhania, R., Wang, Y., Danesh, A., Shen, S.Y., Han, H., Liang, G., Jones, P.A., Pugh, T.J., et al. (2015). DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts. Cell 162, 961-973. 10.1016/j.cell.2015.07.056.
- Wijewardhane, N., Dressler, L., and Ciccarelli, F.D. (2021). Normal Somatic Mutations in Cancer Transformation. Cancer Cell 39, 125-129. 10.1016/j.ccell.2020.11.002.
- Yizhak, K., Aguet, F., Kim, J., Hess, J.M., Kübler, K., Grimsby, J., Frazer, R., Zhang, H., Haradhvala, N.J., Rosebrock, D., et al. (2019). RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. Science 364. 10.1126/science.aaw0726.

yes	x	Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?
no		