GENOME INSTABILITY AND CANCER

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Cancer cells replicate continuously and therefore chemotherapy was designed to hamper DNA replication. However, its success was more limited than what was initially expected both because the treatments do not efficiently kill cancer cells and healthy tissues with high proliferation rates suffer from the chemo-treatment. The knowledge of the signaling pathways that control the cellular response to DNA Damage and the technologies that allow gene editing are powerful tools to improve chemotherapy. In this symposium, we will discuss examples of how basic, preclinical and retrospective studies can provide either alternatives or enhancers of chemotherapy. Such knowledge can be used to combat cancer by designing protocols in the field of precision medicine.

DISSECTION OF MOLECULAR TRIGGERS FOR GENOMIC INSTABILITY AND CELL DEATH IN CELLS TREATED WITH CHK1 INHIBITORS

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Checkpoint kinase 1 inhibitors are been tested in the clinic because they trigger cancer cell death as a consequence of hampering damaged DNA replication. One negative aspect of Chk1 inhibition is the accumulation of chromosome instability, which has been associated with tumor adaptation to the treatment. It is currently accepted that cell death and chromosomal instability are tightly linked because they are both triggered by suboptimal DNA replication in the absence of checkpoint signals. Here we show that bulk DNA replication defects can be recovered after Chk1 inhibition in a manner that correlates with full rescue of cell survival but no elimination of chromosome instability. Conversely, we will also show molecular events that control chromosome instability without affecting the extent of cell death triggered by Chk1 inhibition. Such information may be of use when attempting to improve treatments involving Chk1 inhibitors. Our work may also prompt the search of molecular signals that specifically control genomic instability but not cell death when using other treatments disrupting key DNA damage response pathways which may have or will be transferred to the clinic.

IDENTIFICACIÓN DE BLANCOS MOLECULARES PARA ONCOLOGÍA DE PRECISIÓN EN CÁNCERES BRCA-DEFICIENTES MEDIANTE INDUCCIÓN DE LETALIDAD SINTÉTICA

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Las deficiencias en los genes BRCA1 y BRCA2, ya sea por mutaciones o por expresión aberrante, constituyen un rasgo molecular característico de múltiples tipos tumorales. Con el objetivo de identificar nuevos blancos moleculares para combatir este tipo de neoplasias, en nuestro laboratorio desarrollamos una plataforma de screening basada en citometría de flujo automatizada multiparamétrica, la cual permite la búsqueda simultánea de interacciones de tipo letal sintética en contextos deficientes para BRCA1 o BRCA2. Con esta herramienta llevamos a cabo screenings con diferentes fuentes de compuestos, entre las cuales se desatacan, colecciones de productos naturales nativos de Argentina, bibliotecas de compuestos de empresas farmacéuticas multinacionales y colecciones de inhibidores de kinasas. En este simposio presentaré el desarrollo de la tecnología de screening y me enfocaré en los resultados obtenidos con la colección de inhibidores de kinasas. En particular, mostraré resultados que llevaron a la identificación de la Kinasa PLK1 como un blanco molecular para la inducción de letalidad sintética en células tumorales deficientes en BRCA1. Siguiendo esta línea de resultados, detallaré diferentes modelos de validación utilizados para confirmar este hallazgo y explorar su penetrancia y potencial terapéutico, entre los que se desatacan modelos de xenoinjertos murinos y análisis retrospectivos de bases de datos de pacientes.

HPV E6 AND E7 ONCO-PROTEINS SENSITIZE HUMAN KERATINOCYTES TO OXIDATIVE DAMAGE

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In Uruguay, cervical cancer is the third type of cancer with the highest incidence in women. This tumor is etiologically associated with infection by some types of human papilloma virus (HPV), known as high-risk oncogenic HPV. Persistent infection by these viral types, mainly by types 16 and 18, is associated with the development of severe cervical dysplasia and carcinomas. Cells found in cervical lesions accumulate genetic damage and this is associated with their progress to malignancy and the ability to invade neighboring tissues.

The E6 and E7 onco-proteins encoded by these viruses induce the degradation of p53 and pRB. However, the sustained expression of these onco-proteins is not sufficient for the development of cancer. Therefore, we have studied the degree of sensitization to oxidative damage generated by reactive oxygen species in human keratinocytes transduced with the E6 and E7 genes of HPV 16. *In vitro* assays were performed exposing cells to different doses of hydrogen peroxide. It has been observed that the cells expressing viral onco-proteins E6 and E7 survived after treatment with the oxidizing agent as well as showed a slow repair of DNA damage, which lead to an accumulation of genetic damage evidenced through the analysis of micronuclei. The incorporation of ascorbic acid as an antioxidant agent, showed a reduction of the oxidative damage in cells expressing viral onco-proteins, decreasing the accumulation of genetic damage, suggesting a possible palliative therapy to the detrimental effect of the host inflammatory response produced during HPV infection.

REVEALING TEMOZOLOMIDE RESISTANCE MECHANISMS VIA GENOME-WIDE CRISPR LIBRARIES

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Glioblastoma (GBM) is a severe type of brain tumor with a poor prognosis and few therapy options. Temozolomide (TMZ) has been widely used to treat glioma, however with limited success. TMZ therapeutic failure is mainly due to tumor resistance. The aim of this work was to identify genes that modulate TMZ resistance in GBM. Genome-wide CRISPR-Cas9 lentiviral screen libraries for gene knockout and activation were transduced in human GBM cell line U138MG. Next-generation sequencing was used to identify gRNAs that were enriched in the knockout or activation screen libraries upon TMZ treatment compared to untreated cells. Pathway analysis of gene candidates on knockout screening revealed that mismatch repair and Sonic hedgehog pathway were significantly enriched. Gene silencing of genes ranked on top 10 list (MSH2, PTCH2 and CLCA2) greatly protect the cells from TMZ-induced death. Also, activation genome-wide screen library revealed that NRF2 and WNT pathways are involved on TMZ resistance. Overexpression of FZD6, CTNNB1 or NRF2 was able to significantly increase cell survival upon TMZ treatment. Using TGCA RNA-seq dataset of glioblastoma patients, we confirmed that expression levels of NRF2 and related genes significantly correlate with patient survival rates. Furthermore, several gene candidates from knockout or activation screening are targetable by inhibitors or small molecules, and some of them are already been used in clinics. Overall, our results have identified a number of genes that contribute to TMZ resistance in human glioma cell.
