

TOPOLOGY AND DNA BREAKS GROUP

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OVERVIEW

DNA topoisomerases have a dual relationship with the genome. They are essential to solve supercoiling and other topological problems inherent to all DNA transactions, but their intrinsic mechanism of action can result in the formation of DNA breaks, either accidentally during normal cellular metabolism or upon chemotherapy treatment with the so-called topoisomerase poisons. Imbalances in DNA topoisomerase activity can therefore compromise cell survival and genome integrity, entailing serious consequences for human health, such as developmental and degenerative problems and, very importantly, neoplastic transformation processes and their subsequent response to treatment.

We are interested in understanding how DNA topoisomerase activity is regulated to integrate different aspects of genome dynamics, how an imbalance in these processes can lead to the appearance of pathological DNA breaks, and how cells specifically respond to these lesions to maintain genome stability.

“We have defined a complete map of the genetic pathways operating in the repair of topoisomerase II-induced DNA breaks, their relationships, and how this affects genome stability and tumorigenesis.”

RESEARCH HIGHLIGHTS

During 2022, we had 2 main areas of interest. The first one is in line with the main research line of the laboratory on the repair of topoisomerase II (TOP2)-induced DNA double-strand breaks (DSBs), while the other one is completely different, and stems from the efforts initiated during the COVID-19 pandemic to develop novel genetic diagnosis methods that could be implemented in a point-of-care setting.

Repair of topoisomerase II-induced DNA breaks

TOP2-induced DSBs are particular DNA lesions in which the ends of the break are blocked by a protein adduct that needs to be removed to allow further repair to take place, and can arise spontaneously or as a consequence of chemotherapeutic regimes including TOP2 poisons. We have used unbiased genetic screening approaches to obtain a comprehensive view of the different factors specifically involved in the repair of these lesions. Our results outline 2 main pathways that operate hierarchically to remove the protein adduct (FIGURE 1). First, cells strongly rely on repair mediated by TDP2, an enzyme that directly removes the adduct without affecting the DNA molecule, thus promoting accurate repair and the maintenance of genome stability. Alternatively, but only if this pathway is overwhelmed or disturbed, cells use nucleolytic activities, such as Artemis or the MRN complex, which eliminate the adduct by trimming off DNA ends, allowing repair, but at the cost of compromising genome integrity. As expected from this model, removal of TDP2 in mouse models leads to increased cancer predisposition. Finally, we found that ATM, a common tumour suppressor and the most relevant kinase controlling the response to DSBs, is important for enforcing the hierarchical preference for the TDP2-mediated pathway, and that ATM deficient tumours specifically accumulate mutations that are compatible with the misrepair of TOP2 breaks. Altogether, these results open new avenues to improve the therapeutic use of TOP2 poisons, demonstrate the spontaneous occurrence of TOP2 lesions *in vivo*, and highlight their oncogenic potential when not appropriately repaired.

Novel nucleic-acid detection method

The capacity of CRISPR-Cas systems being programmed to recognise specific nucleic acid sequences has boosted their biotechnological applications. One of them is the detection of the genetic material of pathogens or genetic markers in diagnosis. Systems to detect specific nucleic acid sequences based on CRISPR-Cas technology have been recently developed and promise to revolutionise point-of-care diagnostics in the near future. These systems rely on the fact that, upon recognition and cleavage of the desired target, which is highly specific and easily programmable, the Cas protein becomes activated with a sequence-independent, unscheduled nucleolytic activity that can be easily detected with nuclease reporter substrates, and whose signal can therefore be used as a readout for the presence of the given nucleic acid of interest.

These CRISPR-Cas diagnostics, however, despite their great specificity and versatility, are currently limited by the levels of sensitivity, which are outside the range of the concentrations required for diagnostic purposes, and currently rely on pre-amplification of the target sequences by methods such as PCR or LAMP. This introduces a complication to the reactions, limiting their current use in point-of-care applications. We have developed and patented a conceptually novel solution that, instead of amplifying the target nucleic acid, focuses on boosting Cas activation, so the reaction is carried out in a single step at room temperature, providing an ideal setting for point-of-care diagnostics. Due to its versatility in the detection of any nucleic acid of interest, this invention should constitute the platform for the development of a wide range of specific genetic testing kits and devices, including pathogen and genetic marker detection. ■

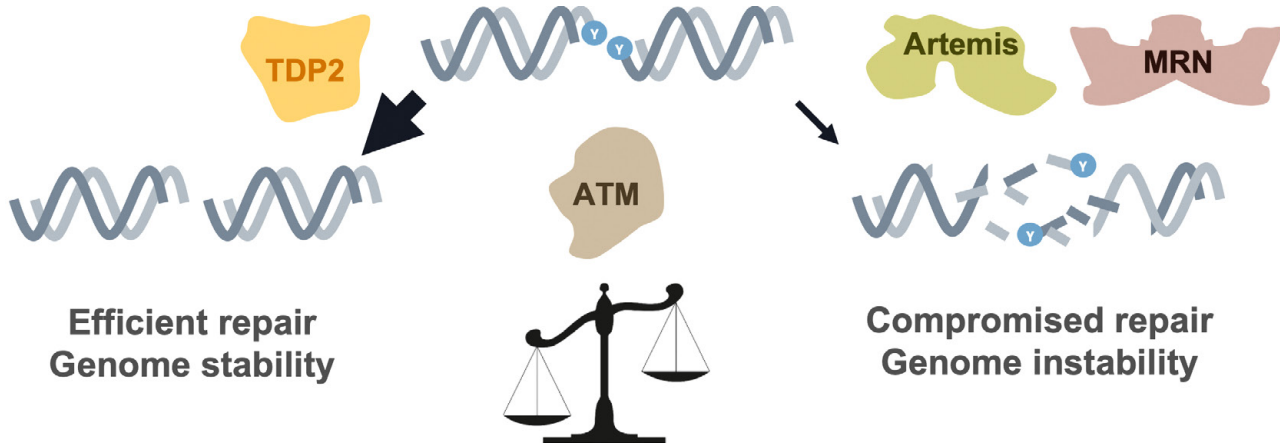


FIGURE 1 Scheme of the different cellular pathways involved in the repair of TOP2-induced DSBs. Protein adducts are removed by either TDP2, which results in accurate repair, or nucleolytic pathways, which compromise genome integrity. ATM establishes a hierarchical preference for the TDP2-dependent pathway.

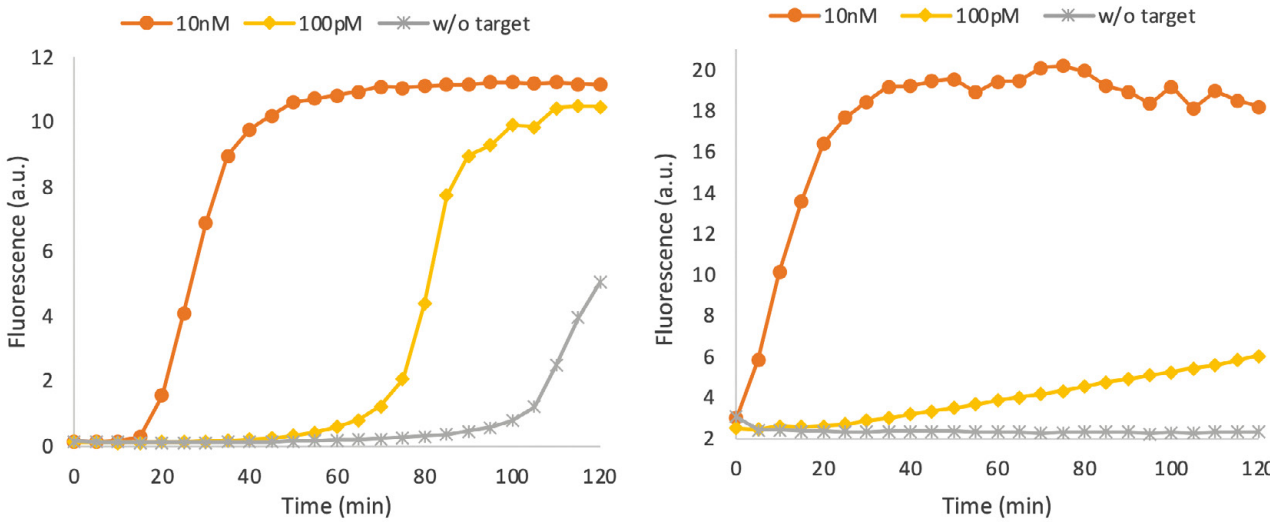


FIGURE 2 CRISPR-Cas12a DNA detection. Our improved method with amplification of Cas activation (left) is compared to direct detection (right).

PUBLICATIONS

Delgado-Chaves FM, Martínez-García PM, Herrero-Ruiz A, Gómez-Vela F, Divina F, Jimeno-González S, Cortés-Ledesma F (2022). Data of transcriptional effects of the merbarone-mediated inhibition of

TOP2. *Data Brief* 44, 108499.
Rodríguez-Cortez VC, Navarrete-Meneses MP, Molina O, Velasco-Hernandez T, Gonzalez J, Romecin P, Gutierrez-Aguera F, Roca-Ho H, Vinyoles M, Kowarz E, Marin P, Rodríguez-Perales S, Gomez-Marín C, Perez-Vera P, Cortes-Ledesma F, Bigas A,

Terron A, Bueno C, Menendez P (2022). The insecticides permethrin and chlorpyrifos show limited genotoxicity and no leukemogenic potential in human and murine hematopoietic stem progenitor cells. *Haematologica* 107, 544-549.

PATENT

Cortés Ledesma F, Gómez Marín C, Muñoz Barrera M, López de Alba E (2022). Nucleic acid detection method. *EP22382608.2*.