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Keywords

Synthetic Biology and Smart Therapeutic Nanosystems Laboratory

Research Lines

- ✓Off-target toxicity limits anticancer treatment efficacy
- ✓Toxin-antitoxin pairs and selective killing of eukaryotic cells
- ✓Synthetic Biology and smart therapeutic systems
- ✓Selective delivery of therapeutic agents to cancer cells

Scientific Activity

The ability of our TA-based synthetic systems to kill specific cancer cells in a highly selective manner has been validated using established cell lines where the expression of specific oncogenes could be induced exogenously, and confirmed using established cells where these oncogenes are constitutively expressed. We will now test our systems using primary cancer and normal cells isolated from human biopsies, both in mixed tissue cultures and in xenotransplanted animals.

The synthetic systems developed by us so far are responsive to the activation of a discrete number of oncogenes in human cells. Our efforts will focus now on the design and fabrication of TA-based systems that respond to a broader range of oncogenic insults, or combinations of them. The objective of this will be, on one hand, to broaden the therapeutic potential of our strategy to a greater number of cancer types and, on the other hand, to be able to use this approach in a personalized manner. In line with this goal we are working on screening methodologies aimed at identifying cs-POMs that turn cytoprotective Kid/Kis ratios into cytotoxic ones in response to new specific genetic alterations and, ultimately, in any diseased cell present in cancer biopsies. We will also investigate whether the co-integration of different cs-POMs into single synthetic systems can be exploited to fine tune differential modulation of Kid/Kis ratios in the highly heterogeneous cellular context of human tumors

We will also direct our efforts at completing the development of a multifunctional DES that we could tailor to different applications, including the intracellular delivery of our TA-based systems to specific cells in vivo. This will include performing preclinical studies in mice to investigate the pharmacokinetic and pharmacodynamic properties of our DES currently under development, as well as the evaluation of their therapeutic index when filled with anticancer agents.

Research lines:

✓Off-target toxicity limits anticancer treatment efficacy

Anticancer drugs must be cytotoxic and selective, so that they eliminate cancer cells rapidly without harming healthy cells in treated patients. Classical chemotherapeutic agents are very potent inducers of cell death but they target molecules that are present both in tumor and normal cells. As a consequence these compounds have poor selectivity and provoke undesired side effects that limit their clinical application.

Other anticancer agents have been molecularly designed to target proteins that are over-produced and/or mutated in cancer cells. Unfortunately, many of these compounds are not selective enough either. This happens because normal cells also produce their target proteins, or because these agents fortuitously target additional proteins that are present in normal cells. Another pitfall of most of these compounds is that they exert only a cytostatic (rather than cytotoxic) effect in cancer cells. This facilitates the selection and expansion of drug-resistant cells and the concomitant appearance of tumors that are refractory to treatment.

Combining cytotoxicity and selectivity in anticancer agents has proved more difficult than anticipated, and this has hampered progress towards effective treatment of the disease. A potential solution to this problem would be to develop therapeutic approaches that rely on the combined action of a cytotoxic agent and cytoprotective agent, and that restrict the function of the latter to healthy cells, so that only they are protected from cell killing during treatment.



✓Toxin-antitoxin pairs and selective killing of eukaryotic cells

Many prokaryotes and their plasmids produce toxin-antitoxin (TA) proteins. For instance, plasmid R1 encodes toxin Kid, a protein that cleaves mRNA at single stranded UUACU sites, and antitoxin Kis, that interacts with Kid and neutralizes it. Kid and Kis function as a plasmid rescue system. The amount of Kis compared to Kid in R1 host cells depends on plasmid copy numbers. In cells where these numbers decrease, the Kis-to-Kid ratio also decreases. This leaves Kid free to act, which arrests bacterial cell growth until the plasmid restores appropriate copy numbers. Once this happens the concentration of Kis increases, re-neutralizing Kid and allowing cells to proliferate again.

Co-evolution of plasmid R1 and its host cells has shaped the specificity with which Kid cleaves RNA, so that activation of this toxin in bacteria only results in a proliferative arrest. In contrast, ectopic expression of Kid induces apoptotic death in eukaryotic cells, an effect from which they are protected by Kis. This offers the possibility of regulating survival and cell death in human cells, simply by controlling exogenously the relative amounts of Kid and Kis that they produce. 'Leaky' behavior of gene expression regulatory elements makes it difficult to direct toxin production exclusively to single cell types in multi-cellular organisms. However, protection of non-targeted cells with appropriate background levels of Kis expression is possible and can be used to refine target cell selectivity, allowing highly selective cell killing in animals.

✓Synthetic Biology and smart therapeutic systems

Synthetic biology is a scientific discipline that integrates knowledge in biology, physics, chemistry, and engineering, and seeks to transform biology in the same way that synthesis transformed chemistry and integrated circuit design transformed computing. It pursues the design and construction of new biological entities (i.e. enzymes, genetic circuits, etc) that can be tuned to meet specific performance criteria. These core components are then assembled into larger integrated systems designed to carry out predetermined functions autonomously and reliably, tailored to solve specific problems.

We are interested in the application of synthetic biology to cancer therapy, in particular the use of toxin-antitoxin pairs to create synthetic systems that are able to distinguish cancer cells from normal cells and take corrective action. The rationale behind our approach is that protein outputs are altered in cancer cells with respect to the normal cells from which they arise. We have built synthetic systems linking Kid and Kis to different cancer-specific Protein Output Modifiers (cs-POMs). The latter are regulatory elements responsible for altering the intracellular concentration of target proteins in human cells exposed to specific types of oncogenic stress. These synthetic systems are able to sense the presence and intensity of such oncogenic signals in the complex and noisy environment of human cells, and respond to it transforming cytoprotective Kid-to-Kis ratios into cytotoxic ones. As a consequence, these 'smart systems' deploy an appropriately tuned, localized therapeutic response that kills targeted cancer cells and keeps non-targeted cells protected from collateral damage.

✓Selective delivery of therapeutic agents to cancer cells

Another way of minimizing off-target toxicity is to administer anticancer agents in an encapsulated form, enclosed in a synthetic container that can be targeted to disease sites, and from which they can escape only when these locations are reached. Drug encapsulation systems (DES) reduce the distribution volume of chemotherapeutic drugs, avoiding indiscriminate exposure of healthy tissues to these agents and reducing the side effects that they cause when administered in their free form. DES also increase the circulation lifetime of encapsulated molecules, and therefore the possibility that they accumulate at disease sites. This is particularly useful when delivering heterologous DNA, RNA or protein molecules, which are immunogenic and rapidly cleared from blood by the reticuloendothelial system in the recipient organism.

We are also developing different systems to encapsulate and deliver TA-based therapeutics to target cells in vivo. To do this, we follow two main approaches, both based in synthetic biology. In some cases, we engineer cells to transform them into factories producing large amounts of vesicles, which can be filled with molecules of different physical and chemical properties, including small chemotherapeutic agents, RNA, DNA and proteins. Further engineering of these cells allows us to modify at will the proteolipidic composition and content of the vesicles that they produce. This enables us to modulate their immunogenicity, target cell selectivity, and the release dynamics and intracellular stability of their cargos. The approach is also used to facilitate the integration of additional functional modules into these DES, to tailor them to specific applications. In other cases, we use synthetic biology to design and produce core components that are then used in a modular way to assemble DES in vitro, enabling the fabrication of bespoke systems, optimized towards particular uses and performance requirements.



Research Projects

- ✓ MBCtheranosticSPION-Development and in vivo validation of a SPION based Theranostic nanosystem for the treatment of Metastatic Breast Cancer, IEF Marie Curie Grant (Manuel Cano Luna), European Comission (2014-2016)
- ✓ Construcción y desarrollo de un nanosistema de diagnóstico multimodal, quelante de Galio y vehiculizable de modo selectivo a células diana in vivo, PI13/02753, Instituto de Salud Carlos III (2014-2016). IP: Guillermo de la Cueva Méndez
- ✓ Desarrollo de un nanosistema terapéutico (nanolPG) para la transducción de células tumorales con pADN o proteínas, y la inducción de su muerte selectiva in vivo, BIO 3120, Consejería de Economía, Innovación, Ciencia y Empleo
- ✓ Desarrollo y validación in vivo de un Nanosistema Teragnóstico basado en Nanopartículas Superparamagnéticas de Óxido de hierro (SPION) para el tratamiento del Cáncer de Mama metastático, PI-0044-2014, Consejería de Ingualdad,salud y políticas sociales (2014) IP: Manuel Cano Luna.

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Patents

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