Intracellular delivery, imaging and drug-sensing using a plasmonic-enhanced hybrid nanostystem

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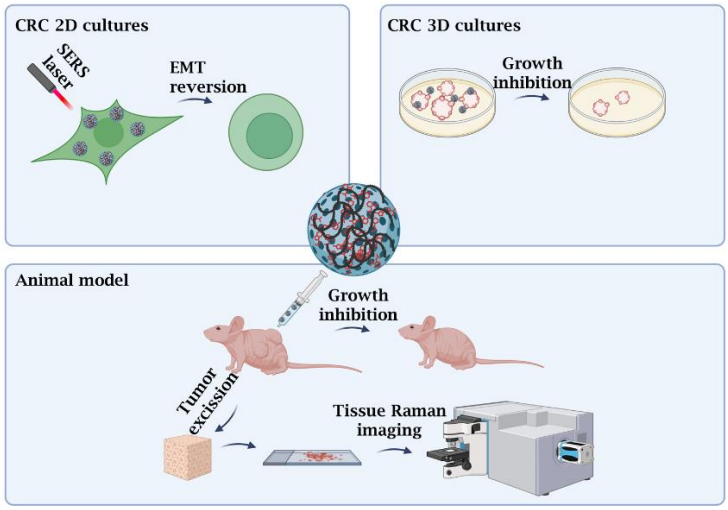
Abstract. Metastasis stands as the leading cause of mortality among colorectal cancer (CRC) patients. Galunisertib (LY2157299, LY) is a small molecule demonstrating promising anti-cancer effects by targeting the Transforming Growth Factor-beta (TGF-β) pathway. This route plays a pivotal role in initiating the epithelial-to-mesenchymal transition (EMT), a critical process for metastatic spread. Unfortunately, LY chronic treatment causes undesired effects. To mitigate these side effects, nanoscale drug delivery systems have emerged as a transformative approach in cancer treatment, enhancing drug effectiveness while minimizing toxicity. In this study, we introduce a hybrid nanosystem (DNP-AuNPs-LY@Gel) comprising porous diatomite nanoparticles decorated with plasmonic gold nanoparticles (AuNPs), encapsulating LY within a gelatin shell. This multifunctional nanosystem demonstrates efficient LY delivery, EMT reversal in CRC 2D and 3D cultures, and anti-cancer effects in vivo. Moreover, the nanosystem allowed the quantification with sub-femtogram resolution of the drug intracellularly released using surface-enhanced Raman spectroscopy (SERS). The release of LY is triggered by CRC cell acidic microenviroment. Real-time monitoring of drug release at the single-cell level is achieved by analyzing SERS signals of LY within CRC cells. The heightened efficacy of LY delivery through the DNP-AuNPs-LY@Gel complex offers a promising alternative strategy for reducing drug dosages and subsequent undesired effects.



Graphical abstract. Nanoparticles, encased in a pH-dependent gelatin layer and loaded with the drug, were employed in both 2D and 3D cultures of colorectal cancer (CRC), demonstrating a significant anti-tumoral effect. Furthermore, we successfully quantified the intracellular delivery of the anti-cancer drug using Surface-Enhanced Raman Spectroscopy (SERS). Subsequently, the nanosystem underwent testing in an animal model, revealing notable inhibition of tumoral growth. Notably, the hybrid nanosystem enabled label-free imaging of the nanoparticles within the tumoral tissue through Raman microspectroscopy.

1 Introduction

Colorectal cancer (CRC) stands as one of the foremost causes of mortality globally [1]. In the context of CRC metastasis, Transforming Growth Factor-beta (TGF-β) signaling plays a pivotal role in promoting the epithelial-mesenchymal transition (EMT), a crucial step in initiating metastasis. In this context, targeting TGF-β emerges as an attractive strategy for developing novel therapies. Galunisertib (LY2157299, LY) is a small molecule that acts on the TGF-β pathway and has been proposed as a promising tool to treat metastatic CRC [2]. Unfortunately,

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chronic treatment with LY leads to several unsafe and undesired effects [3]. Here in, we present the development and characterization of a new nanovector composed of diatomite and gold nanoparticles (DNPs-AuNPs) carrying LY. DNPs stand out as optimal candidates for nanovector production due to their remarkable biocompatibility, demonstrating minimal toxicity and preserving cell viability effectively [4]. The nanovector is encapsulated within a pH-dependent gelatin (Gel) matrix, ensuring drug release specifically within cancer cells characterized by an acidic pH. Furthermore, the nanovector is decorated with gold nanoparticles (AuNPs), enabling label-free quantification of LY delivery inside CRC cells via Surface Enhanced Raman Scattering (SERS) [5-7]. which facilitated the quantification of intracellular LY delivery at sub-femtogram resolution, allowing correlation between the amount of internalized drug and its therapeutic effect. Our study highlights the potential of this novel nanovector as a targeted and effective approach for the treatment of metastatic CRC, offering insights into its mechanism of action and therapeutic outcomes.

2 Results and Discussion

DNPs-AuNPs were synthesized following the procedure previously described [5]. The size distribution of the DNPs was analyzed using Dynamic Light Scattering (DLS), revealing a mean diameter of 400 ± 50 nm. Subsequently, LY was loaded into the nanovector by incubating the drug with DNPs-AuNPs in an acidic solution to facilitate electrostatic interactions between LY and the nanovector surface. Finally, the DNPs-AuNPs-LY nanovector was encapsulated with a gelatin (Gel) layer, crosslinked using carbodiimide chemistry. The loading capacity (LC) was calculated as previously described and resulted to be of 20 μg of LY per milligram of DNPs [3]. Furthermore, the release profile of LY from the nanovector was examined in both acidic and basic environments. As expected, LY was efficiently released in the acidic solution, while negligible release was observed in the basic environment. The preliminary study of the drug release in vitro is crucial for understanding the nanoplatform behavior in varying pH conditions. Nevertheless, in vitro release tests do not fully replicate the complex cellular environments. To overcome this limitation, we decorated the nanovector surface with AuNPs that allows us to monitor and quantify the real-time release of LY directly within CRC cells by SERS [35]. LS.174T, used as model of CRC cells, were incubated with DNPs-AuNPs-LY@Gel and the LY-SERS signal was monitored at different times. After 18 hours, approximately 30% of the total LY was delivered into the cells. Given that each DNP unit contained 0.25 femtograms (fg) of LY, the amount of LY delivered by a single DNP into the cell was estimated to be 0.075 fg. Subsequently, the released LY increased to 50% after 24 hours. Our nanovector allowed an efficient SERS-based intracellular drug tracing, sensing, and quantification within living CRC cells, achieving a sensing resolution as low as 7.5×10^{-18} g. The nanovector therapeutic efficacy was tested in LS.174T 2D and organoid-like (*i.e.*, 3D) cultures. All the experiments were also conducted with LY alone, to assess if the nanoplatform was able to enhance the LY efficacy. Given the LY ability to block the TGF β receptor signalling pathway, we examined the impact of the hybrid system on the expression levels of key genes involved in EMT. Encapsulating LY within the nanoplatform bolstered its metastasis-blocking potential by downregulating pro-metastatic genes in both 2D and organoid-like cultures. Moreover, the treatment with the hybrid nanoplatform prompted significant morphological changes in cells grown in monolayer within 48 hours, transitioning from elongated and spindle-shaped to rounded forms, signifying reduced aggressiveness and wildtype characteristics. Nanovector treatment of organoid-like culture of LS.174T cells caused a reduced growth resulting in a minor dimension and number of the organoids. These findings underscore the potency of LY when encapsulated within the hybrid nanoplatform, demonstrating an efficacy over three times greater than conventional drug administration. Ongoing investigations are underway to assess the biocompatibility of the nanovector, with a focus on validating its efficacy in vivo. Preliminary data suggest promising potential for in vivo applications, particularly in influencing tumor growth and progression. Furthermore, current research efforts are directed towards refining the nanovector specificity towards CRC cells through the implementation of an active targeting strategy.

3 Conclusions

This study allowed the synthesis and characterization of an innovative nanovector, which exhibit efficient drug delivery capabilities and therapeutic efficacy in CRC cells. Overall, our findings highlight the promising application of DNPs-AuNPs-LY@Gel nanovectors as a targeted and effective therapeutic strategy for CRC treatment.

This work was supported by: AIRC Bridge Grant #27012, Young Researchers-MSCA-PNRR-MUR # MSCA_0000023 and PNC 0000001_D3 4 Health to E.L.; AIRC IG grant # 21420 to A.C.D.L.; FIMP and Fondazione Umberto Veronesi to D.D.C. This work is partially supported by the NextGenerationEU Project PNRR-SEE LIFE - Strengthening the Italian Infrastructure of Euro-Bioimaging (IR23 - DD MUR 3264/21 CUPB53C22001810006)

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