Editorial

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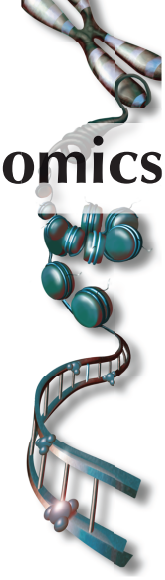

Combination therapy and noncoding RNAs: a new era of cancer personalized medicine

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> "This peculiarity of lncRNAs and miRNAs, of creating a noncoding 'interactome' to regulate molecular pathways, paves the way to multiple approaches to target tumor resistance with ncRNA-based combination therapies"

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The emergence of novel therapies designed to overcome treatment resistance and improve patient outcomes is often counteracted by drug resistance, which still represents a major cause of reduced quality of life and poor survival for cancer patients. Each patient and disease are different because of the intricated molecular mechanisms and changes that may occur at a genome, epigenome and transcriptome level. Therefore, a new era has opened for cancer treatment, based on personalized medicine. If researchers could track the molecular mechanisms of cancer treatment resistance and recurrence after therapy, these responses could be anticipated by combination treatments, to simultaneously turn off pathways of primary cancer growth and of later relapse. In the present day, personalized medicine could be particularly efficacious via combining existing approved treatments with novel treatment approaches, designed for more specific therapeutic targets, such as noncoding RNAs (ncRNAs). ncRNAs are transcripts lacking protein-coding potential, but they can have key cellular functions, from the recruitment and stabilization of epigenetic effectors and transcription factors to the modulation of alternative splicing and other post-transcriptional roles. For instance, via their interactions with epigenetic complexes, ncRNAs can regulate DNA methylation and histone modifications, thereby promoting chromatin remodeling, and the extensive control of gene expression.

The most characterized ncRNAs in cancer are miRNAs and long ncRNAs (lncRNAs). As ncRNAs are often upregulated in specific conditions, such as cancer subtypes, and are often found aberrantly expressed in cancer, they have been recently in the focus as novel promising targets for cancer combination treatments [1,2]. Targeting ncRNAs in combination therapies as first or second line treatment could have an enhanced effect on tumor growth and drug resistance inhibition [3].

A study from almost a decade ago shed light on ncRNAs as drivers of cancer aggressiveness and their synergistic combinatorial therapeutic potential in cancer [4]. In fact, Pencheva *et al.*, showed that *miR-1908*, *miR-199a-5p* and *miR-199a-3p* trigger metastasis in melanoma via the dual inhibition of ApoE, suppressor of metastasis and angiogenesis in this cancer, and the heat shock factor DNAJA4, which induces ApoE expression [4]. They used locked nucleic acids (LNAs) to target these miRNAs, thereby strongly suppressing cancer metastasis [4]. LNAs are antisense oligonucleotides (ASOs) with chemical modifications, which increase the stability and strength of hybridization to target RNA molecules. ASOs are 13–200 nts single strand nucleic acids, designed to complementary bind endogenous RNAs, thereby recruiting the RNase-H enzyme to degrade them. As RNase H is active in both cellular cytoplasm and nucleus, ASOs effects are often successful to target ncRNAs [5,6]. An example is a study on long noncoding RNA *TUG1* in pancreatic ductal adenocarcinoma, where this lncRNA expression is prognostic of overall survival in patients treated with 5-FU-based treatment, which represents the standard chemotherapy for this cancer [5]. *TUG1*-targeting ASOs sensitize resistant cells and *in vivo* models to 5-FU, with the combination treatment reducing tumor growth significantly, compared to 5-fu alone [5]. As this model shows that *TUG1* inhibits

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miR-376b3p [5], additional studies could prompt the use of *miR-376b3p* mimics to enhance the combination treatment effect.

This peculiarity of lncRNAs and miRNAs, of creating a noncoding 'interactome' to regulate molecular pathways, paves the way to multiple approaches to target tumor resistance with ncRNA-based combination therapies; in fact, in cases where lncRNA-targeting ASOs are not well tolerated, or not inducing enough response, miRNA mimics could be used to achieve the same result or potentiate the ASOs effects. These multifaceted roles of ncRNAs are reflected in higher possibilities to find molecular targets for the mechanisms that they trigger, with lower side effects and increased chances of targeting success. Additionally, despite the fact that ncRNA-based treatments are under-investigated compared to protein inhibitors, due to their more recent discovery and characterization, several other studies show that multiple options can be adopted to effectively target ncRNAs in combination therapies, such as CRISPR-Cas9 or more complex nano-formulations [7–11].

CRISPR-Cas9 has been used to target lncRNA *UCA1* and *PD-1*, as effective combination therapy in bladder cancer, where previous targeted therapy gave effective but short-term response [7]. Knockout of *UCA1* and *PD-1* inhibits tumor growth and increases survival in xenografted models, with potentiated effect due to the immune checkpoint blockade of PD-1, determining the upregulation of interleukins and cytokines and activation of interferon gamma-dependent PD-L1 expression [7]. The immune and antitumor cascade activated by the co-targeting of *UCA1*, and PD-1 consequently reshapes the immune microenvironment from an immunosuppressive to a stimulatory state, with an effective and potentially prolonged treatment. CRISPR-Cas9 is a recent and promising technology in cancer research but its use in patients could be critical compared to other ncRNA-based therapeutic systems.

Nanoparticle's technology and ncRNAs have demonstrated promising uses for cancer combination therapies. Multiple nano-formulations can be obtained and can be adapted to the cancer, the molecules to be carried and multiple other factors, to maximize the delivery efficiency and minimize the side effects and toxicity. A study has used a combination of *miR-34a* and siRNA targeting oncogenic *KRAS* carried by a new lung-targeting nanoparticle, in lung adenocarcinoma cells and mouse models [8]. This system results in reduced *KRAS* gene expression and MAPK signalling, increased apoptosis, and inhibited tumor growth, with overall increased response, compared to the agents alone [8]. Notably, the triple combination of these nanoparticle-mediated small RNAs delivery with cisplatin-based chemotherapy prolongs *in vivo* survival compared to chemotherapy alone [8]. A more recent study has investigated the use of polymeric hybrid micelles, engineered to co-load irinotecan and *miR-34a* [9]. Irinotecan is a chemotherapeutic used in colorectal cancer, but it is not effective for advanced metastatic stages; *miR-34a* is a tumor suppressive miRNA which could enhance irinotecan effect at later cancer stages. The use of these particles *in vitro* has a cytotoxic effect on cancer cells with increased apoptosis [9]. Intravenous administration of this combination therapy *in vivo* has extraordinary antitumor efficacy and good biocompatibility [9]. In a recent study, nanoparticles modified with one mitochondrion-directed peptide and a tumor-targeted ligand are used to effectively deliver *miR-125* and the natural phytochemical Ellagic acid, effective on mitochondrial disfunction but poorly stable and soluble in biological fluids [10]. This system successfully targets proteins involved in mitochondrial dynamics of tongue squamous cell carcinoma cells and decreases tumor growth *in vivo* [10]. LncRNAs can also be useful targets in combination to natural compounds to treat cancer using novel nano-formulations [11]. In fact, formulation of polymeric hybrid nanoparticles has been used in colorectal cancer to deliver curcumin molecule together with a siRNA targeting the lncRNA *CCAT1*, characterized as oncogenic in several cancers [11]. This combination treatment results in the inhibition of proliferation and migration *in vitro* and of tumor growth in patient-derived xenograft models of colorectal cancer, with good biosafety and biocompatibility [11].

A different and recently challenging ncRNA-based approach to target cancer cells with combination treatment is via stimulus-responsive drug release [12]. Two onco-miRNAs, *miR-21* and *miR-10b*, upregulated in metastatic breast cancer cells, have been used to activate the release of a combination treatment, consisting of doxorubicin and a siRNA targeting *BCL2* mRNA [12]. This complex is made with gold nanoparticles carrying two pairs of released DNA/RNA duplex hybridized to generate the siRNA *in situ*, by strand displacement reactions, and doxorubicin molecules, located in the duplex intercalating sites [12]. The system is activated by the two miRNAs, catalysing the disassembly of the nanocarrier to release doxorubicin and the DNA/RNA hybrids that spontaneously hybridize to create the *BCL2* targeting double stranded siRNA [12]. Herein, miRNAs are used as trigger of co-treatment release, specifically in cancer cells, where doxorubicin inhibits cell division and growth, and the siRNA-based targeted therapy inhibits BCL2 oncoprotein translation [12]. As ncRNAs have been successfully studied as diagnostic and prognostic biomarkers and some are also in clinical trials or approved in cancer [13,14] (NCT01024959 [15]), their use

as trigger of drug release, specifically to cancer cells, paves the way for the formulation of new effective treatments with lower side effects for healthy cells. This is particularly important for chemotherapy and other treatments, which do not specifically target cancer cells, resulting in high toxicities for patients compared to the actual drug benefits.

Noncoding RNAs are also well characterized to simultaneously regulate different pathways in the same cellular condition, thereby triggering a multitude of downstream molecular mechanisms with possible synergistic effects [16]. This peculiarity can be exploited by creating either ncRNA-based formulations for combination therapies as discussed so far, or potential phenotype-based ncRNA screening approaches to identify novel combination therapies [16]. An example of the latter is the case of miR-3622b-5p, which has cytotoxic effects in chemo resistant ovarian cancer cells, found using gain-of-function miRNA screening with real-time continuous cell monitoring [16]. *MiR-3622b-5p* targets Bcl-xL and EGFR-mediating BIM upregulation, thereby inducing apoptosis in the resistant cells and resulting in cisplatin sensitization [16]. In the same cancer, *miR-3622b-5p* can inhibit cell migration via LIMK1 and NOTCH1 inhibition. In this study, the authors have used *miR-3622b-5p* multi-targeting ability to investigate protein-based combination treatments using an EGFR inhibitor together with a BH3-mimetic molecule, to prompt a significant inhibition of ovarian cancer cells and organoids viability. In this context, the use of *miR-3622b-5p* mimics, also as a treatment for ovarian cancer, could result in stronger anticancer potential with reduced side effects compared to a protein-based targeted therapy.

All these studies highlight a wide range of approaches involving ncRNAs into combination treatments. A fundamental factor for the implication of ncRNAs in all these uses is the multitude of cellular functions that they can regulate in cancer and in the surrounding tumor microenvironment. Hence, ncRNAs give also the possibility to target multiple downstream targets which can create simultaneous and synergic responses but also create novel targets, where the downstream genes are difficult to target [17]. An example is the *MYC* oncogene located on chromosome 8q24 and co-gained with adjacent genes including lncRNA *PVT1* in colon cancer. Increased *PVT1* expression stimulates MYC protein expression levels in 8q24-amplified human cancer cells [17]. These findings can help targeting MYC-driven colon cancer when MYC is refractory to small molecule inhibition. In fact, CRISPR/cas9 mediated knock out of *PVT1* in MYC-driven colon cancer cell line highly decreases tumor growth *in vivo* [17]. Despite CRISPR-Cas9 has been successfully used *in vitro* and *in vivo*, several limitations arise for its use in patients, including possible risks associated with off-target editing. This study, as most studies on ncRNA-based formulations, highlights their successful use in preclinical models, but only a minority of them reaches a patent stage, through which they could gain a stronger translational potential. A different approach could be to investigate the most successful ncRNA-formulations in multiple studies and different cancers to estimate the actual benefit that their translational potential could have for patients. This approach could bring ncRNA-based combination therapies closer to a clinical reality. ncRNA-based combination therapy can be used for a wide range of approaches to increase treatment effectiveness and specifically target cancer cells or even specific cancer subtypes. Therefore, their use in the clinics could minimize treatment side effects and toxicities, in favor of the actual therapy benefits, paving the way for a new chapter of cancer personalized medicine.

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