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Special issue: Tackling therapy resistance in cancer

Spotlight

Enhancing oncogenic signaling to kill cancer cells

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Cancer-targeted therapies that inhibit oncogenic signaling often lead to resistance and recurrence. In a recent study, Dias *et al.* propose activating oncogenic pathways and inducing replication stress, resulting in cell death and tumor-suppressive mechanisms in colorectal cancer (CRC). This approach could spark a new wave of target discovery, and drug development and repurposing against cancer.

In recent decades, cancer-targeted therapy has focused solely on inhibiting oncogenic pathways [1,2]. Despite short-term benefits, almost all treated patients develop resistance and experience cancer recurrence [1,3]. Yet, a large body of evidence suggests that human cells, especially cancerous ones, can be vulnerable to specific pathways overactivation, particularly the **extracellular signal-regulated kinase (ERK) pathway** (see Glossary) [1–4]. At least 40% of cancers involve ERK pathway aberrations [3]. This susceptibility has been primarily overlooked in previous drug development efforts. However, two noteworthy exceptions exist: the development and preclinical exploration of ERK pathway activators [4] and **KRAS** agonists [5] as potential antineoplastic agents. Additionally, while cancer cells have impaired **DNA damage (DD)** responses due to genetic alterations, they retain mechanisms to counter **replication stress (RS)** subsequences [6]. As such, tumor cells avoid lethal events, such as mitotic catastrophe, due to unresolved stress [6]. Owing to these discoveries, targeting RS tolerance has become a new therapeutic concept in cancer [6]. Bernards and Dias previously proposed that cancer cells exhibiting heightened ERK pathway activity and elevated basal RS levels are selectively susceptible to hyperactivation of these pathways [1].

In a groundbreaking study, Dias *et al.*, from Bernards laboratory, introduced a novel combined treatment for CRC and beyond by activating the ERK pathway and inducing RS [7]. What sets apart their study from previous studies is the authors' focus on combining the overactivation of oncogenic signaling pathways and perturbation of the resulting stress responses as a novel therapeutic strategy (Figure 1).

As a primary target, Dias *et al.* chose protein phosphatase 2A (PP2A), a key regulator of cell growth and survival pathways that exhibits varied roles in cancer, including tumor-suppressor functions [7]. PP2A has several targets and shows a dual effect on ***CRAF***, one of the effectors of the ERK pathway [8]. PP2A not only activates CRAF by dephosphorylating serine 259, but also returns it to an inhibited state by dephosphorylating several other residues [8]. As such, PP2A is known as a negative regulator of CRAF overall during the ERK pathway negative feedback loop [8].

Dias *et al.* tested the clinically relevant PP2A inhibitor LB-100 on seven CRC cell lines with diverse genetic backgrounds, allowing them to assess the broad effects of PP2A inhibition across different genetic profiles. Besides observing moderate toxicity in all cell lines, they determined whether LB-100 could induce the intended oncogenic signaling [7]. They treated two CRC cell lines (HT-29 and SW480) at sublethal drug doses at different time points. RNA-sequencing and bioinformatics analyses revealed positive enrichment in gene sets related to the ERK and **WNT** pathways, which regulate cellular processes including proliferation, survival, and differentiation, as well as genes associated with DNA damage and **apoptosis** [7]. They performed **CRISPR activation and knockout screens** in HT-29 and SW480 cells, targeting most genes. Aiming to find potential co-targets with LB-100, they identified that upregulating proto-oncogenes, such as MYC and MAP3K1, enhanced LB-100 toxicity, while knocking out genes in the ERK and WNT/β-catenin pathways mitigated this toxicity [7].

Glossary

Apoptosis: a type of programmed cell death in metazoans, including human cells. Negative regulation of apoptosis is a hallmark of cancer.
***CRAF*:** encodes a protein that is an effector of the ERK signaling pathway.
CRISPR activation and knockout screens: genetic screens based on a CRISPR/Cas9 gene-editing system to knock out or activate a set of desired genes in cells. Gene knockdown can also be performed.
DepMap portal: public database affiliated with the Broad Institute that provides genetic and pharmacological perturbation data of cancer cell lines (Cancer Cell Line Encyclopedia).
DNA damage (DD): damage to the DNA molecule that can lead to mutations and loss of genomic instability; can be caused by extrinsic or intrinsic stress signals and events. Cancer cells often have impaired DD response pathways.
Extracellular signal-regulated kinase (ERK) pathway: signaling pathway that governs major cellular events, such as proliferation and survival. The ERK pathway is also known as the mitogen-activated protein kinase (MAPK) or Ras-Raf-MEK-ERK pathway.
***KRAS*:** encodes a protein that functions upstream of RAFs (such as CRAF) that is frequently mutated in cancer.
Replication stress (RS): scenario that occurs when the DNA replication process and replication fork are challenged or stalled. Cancer cells have higher levels of RS.
WEE1: 'Wee' in the Scottish dialect means little or small. Paul Nurse, of the University of Edinburgh, named the discovered protein as such. The kinase protein impacts cell size due to its regulation of function during the cell cycle.
WNT: cellular signaling cascade that significantly functions in embryogenesis, cell homeostasis, and stem cells.

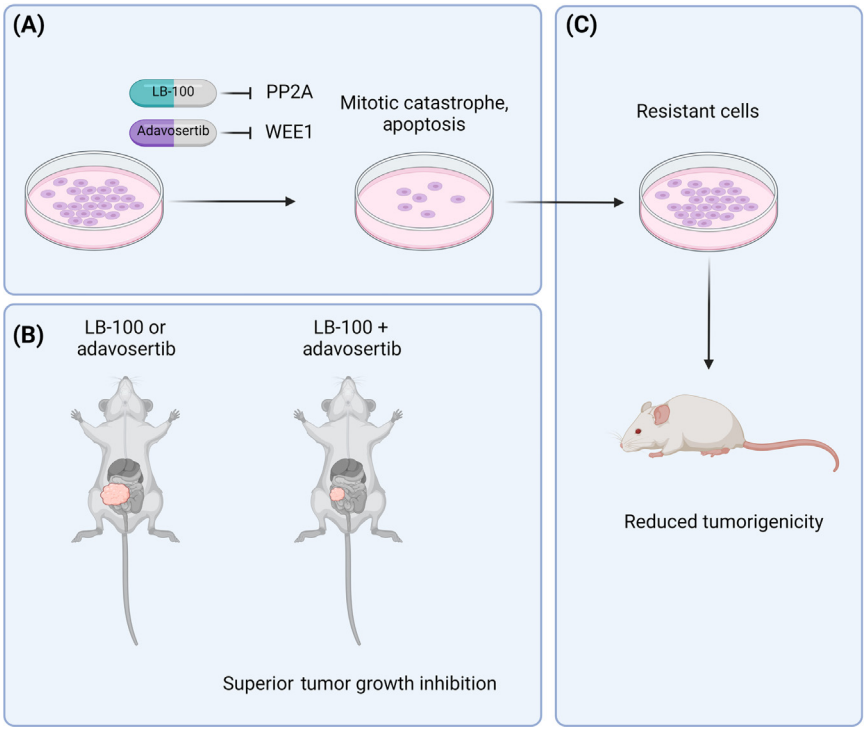

Figure 1. Overview of the synergistic effects of LB-100 and adavosertib *in vitro* and *in vivo* on colorectal cancer (CRC) cells [7]. (A) LB-100 and adavosertib inhibit protein phosphatase 2A (PP2A) and WEE1, respectively, leading to increased oncogenic signaling, such as through the extracellular signal-regulated kinase (ERK) pathway, inducing replication stress (RS). This results in mitotic catastrophe and apoptosis in CRC cells. (B) Colon-implanted patient-derived xenograft (PDX) mice treated with the combination of LB-100 and adavosertib exhibited enhanced tumor inhibition compared with either single agent alone. (C) CRC cells from (A) were treated for 4 months with LB-100 and adavosertib to generate cell lines with acquired resistance. Compared to control cells, these resistant cells exhibited tumor-suppressive features *in vitro*, while also displaying less aggressive phenotypes and diminished cancer-related characteristics. When transplanted into the flanks of mice, these resistant cells also exhibited reduced tumorigenicity. Figure created using BioRender (biorender.com).

While differentially expressed genes provide insights into potential co-targets for drug development, drug screens offer a more direct path toward repurposing available compounds [9]. Dias *et al.* assessed the synergy between a library of 164 drugs that act on cellular stress response pathways and a sublethal dose of LB-100 in two cell lines, identifying **WEE1** and CHK1 inhibitors to be synergistic with LB-100. They then focused on the more clinically advanced WEE1 inhibitor adavosertib [7]. It was previously known that blocking WEE1 hampers the G2/M checkpoint, pushing S-phase cells into premature mitosis during cell division [7]. A genome-wide CRISPR knockout screen reaffirmed WEE1 as a co-target with LB-100 at a lower dose. The LB-100 and adavosertib combination showed synergistic effects in various cancer models but not in non-malignant cells [7]. The combination also significantly prolonged mitosis, causing defective chromosome alignment, mitotic catastrophe, and elevated apoptosis in HT-29 cells. This combination also induced widespread and severe DD, increased single-stranded DNA foci indicating replication stress, and premature mitotic entry with inadequate DNA synthesis [7].

However, adavosertib may cause dose-related adverse events [10]. To evaluate the *in vivo* efficacy and safety of the LB-100 and adavosertib combination, the authors assessed its antitumor effects in three colon-implanted patient-derived xenograft (PDX) models and one cholangiocarcinoma PDX in immunocompromised mice. The combination treatment showed statistically significantly superior antitumor effects compared with single agents in all models except one, which only trended toward enhanced effects. Postmortem analysis revealed no signs of toxicity in vital organs [7].

Mainstream cancer treatments often lead to upregulation of oncogenic pathways and increased RS in resistant cells, resulting in a more aggressive phenotype [1,3,6,7]. However, Dias *et al.* found a different scenario with the LB-100 and adavosertib combinatorial treatment. Resistant cell lines showed a decrease in oncogenic signaling molecules, such as β-catenin, JUN, MYC, and E2F, as well as reduced RS and aneuploidy, compared with parental cells. Single cell analysis revealed that resistant subclones predominantly downregulated these oncogenic pathways, unlike typical therapies [7]. Remarkably, the resistant cells exhibited reduced anchorage-independent growth and failed to induce significant tumor growth in mice. As such, Dias *et al.* report striking tumor-suppressive resistance mechanisms through the paradoxical activation of oncogenic pathways as a therapeutic strategy [7].

Unlike LB-100, the narrow therapeutic window of adavosertib warrants careful patient selection based on toxicity biomarkers before it can be explored further in combination therapies [7,10]. The fate of implementing this combinatorial treatment and similar approaches in clinical practice is no subject of prophecy. Nonetheless, a novel class of experimental drugs has emerged [4,5,7]. These agents, irrespective of their direct pharmacological effects, aim to damage cancer cells by paradoxically activating oncogenic pathways [1,3]. The highlighted study [7] and previous works on novel oncogenic agonists [3] open a new avenue

for target discovery, drug design, and repurposing. Genetic screens are powerful tools for finding new therapies, including through drug repurposing. However, before embarking on experimental work, revisiting publicly available data sources, such as the **DepMap portal**, could help uncover overlooked targets for oncogenic pathway hyperactivation and RS induction.

This nascent field faces the challenge that such paradoxically activating therapies, although selectively harmful to cancer cells, may still risk being carcinogenic to normal cells. Thus, more data are needed to assess the validity of this concern properly.

Declaration of interests

No interests are declared.

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