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Morning for irofulven, what could be fiNER?

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Summary

Cancers with DNA repair dysfunction are vulnerable to DNA damaging agents that invoke a requirement for the disabled repair mechanism. Genome sequencing, coupled with a detailed understanding of mechanisms of DNA repair, has accelerated the discovery of pathway-selective agents that target DNA repair deficiencies in a tumor tissue-agnostic manner.

In this issue of *Clinical Cancer Research*, independent reports from the Szallasi and Offit groups describe the exciting discovery that cancers harboring mutations within the nucleotide excision repair (NER) pathway can be selectively targeted by small molecules that invoke a need for this repair mechanism. Börcsök(1) and Topka(2) provide compelling results that a previously discarded anti-cancer agent, irofulven, offers potential advantages over currently standard treatments for the subset of cancers where nucleotide-excision-repair (NER) mutations are prevalent.

How to selectively target cancer cells by exploiting alterations in their endogenous genetic circuits has long been a central question in cancer therapy(3). Alterations in DNA repair capacity represent a particularly promising vulnerability that creates predictable responses to targeted therapies irrespective of the tissue of origin. This is a consequence of prevalent germline and somatic DNA repair gene mutations in specific cancer types, and because similar mechanisms exist to repair the myriad genomic lesions that arise in all dividing cell types.

Modern approaches to molecularly classifying tumors typically involve genome sequencing coupled with sophisticated computational analyses(4). Targeted exome sequencing alone can detect loss of function mutations in DNA repair genes. Alternatively, DNA repair deficiency can be inferred from specific mutational patterns observed when genome integrity processes go awry(4). This indirect means of determining DNA repair dysfunction from genomic scars is important given the incomplete understanding of how repair mechanisms become dysfunctional in cancers.

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Armed with these tools, investigators are presented with the question of how to prosecute knowledge of DNA repair mechanism inactivation in cancers. A now validated approach comes from the concept of synthetic lethality. This phenomenon was first described in experiments in fruit flies that indicated combinations of alleles in two non-essential genes resulted in lethality(5). Simply put, loss of gene A or B alone does not affect viability but combining inactive A and B alleles is lethal. Synthetic lethality was later proposed as a strategy to treat cancer(3), based on genetic screens in yeast that identified lethal loss-of-function gene combinations or sensitivities to commonly used genotoxic therapies. This degree of unbiased screening is now possible in human cells due to advances in genome engineering technologies(6).

Synthetic lethality was first implemented for cancer chemotherapy in cells harboring loss-of-function *BRCA* mutations. The *BRCA1* and *BRCA2* genes encode proteins that are vital to homologous recombination (HR) DNA repair. Heterozygous germline mutations in *BRCA1* and *BRCA2* account for the majority of hereditary breast and ovarian cancer susceptibility syndromes. Non-cancerous cells in a patient contain one normal *BRCA* allele and have intact HR, whereas tumors inactivate HR repair due to loss of the wildtype *BRCA* gene. Poly(ADP)ribose polymerase inhibitors (PARPi) were shown to be selectively toxic in cells with mutations in both *BRCA* alleles, while being well-tolerated when one wildtype allele is present(7). Clinical efficacy in HR deficient cancers was subsequently demonstrated in multiple tumor types, with four PARPi now being FDA approved for ovarian, breast, and prostate cancers.

The success of PARPi has led to surging interest in identifying additional vulnerabilities in cancers with DNA repair deficiencies. The reports from Börcsök and Topka represent an important advance in this arena. Germline and somatic mutations to NER genes are common in bladder cancer and predict improved responses to platinum chemotherapy. However, resistance is a major limitation as platinum adducts can be removed by several other repair pathways, including by *BRCA*-dependent HR (Figure). Co-morbidities also preclude platinum use in many patients, emphasizing the need for more specific agents that directly attack the underlying deficit in NER.

Irofulven (hydroxymethylacylfulvene) is a semi-synthetic derivative of illudin S that had been previously shown to cause toxicity in cells with impaired NER(8). Global genome-NER (GG-NER) and transcription coupled NER (TC-NER) represent related pathways that remove bulky DNA adducts. TC-NER is mediated by the TF-IIH complex, which activates NER at transcription blocking lesions. Following adduct recognition, the ERCC2 and ERCC3 helicase components unwind DNA, followed by endonuclease mediated incision on either side to remove the adduct. DNA synthesis then fills in the resultant single stranded DNA gap (Figure). Irofulven is believed to generate bulky DNA base adducts that had been shown to cause lethality specifically in cells with impaired TC-NER but not GG-NER. In unselected patient populations irofulven had disappointing results.(9) The work presented in this issue suggests irofulven might be clinically more effective if administered specifically to patients who have tumors with impaired TC-NER..

Both groups used genome engineering approaches to show that irifolven toxicity occurred in cells harboring *ERCC2* or *ERCC3* mutations. Hypersensitivity to irifolven was observed in cells with common *ERCC2* variants and in mammary epithelial cells engineered to contain only one mutant *ERCC3* allele. Importantly, resistance to platinum agents did not mitigate irifolven toxicity in NER mutant cells. Each study also provided unique insights. Börcsök and colleagues (1) created a logistic regression model named *ERCC2mut* classifier based on TCGA bladder cancer exome sequencing (WES) data sets. Encouragingly, the algorithm may predict patients who have NER deficiency not limited to *ERCC2* mutation. It could also be used to find tumors that respond favorably to platinum chemotherapy. Another advance comes from Topka, Offit, et al. (2), who show synergy between irifolven and PARPi as well as other agents in NER-mutant cancers. The sophisticated prediction pipelines and new combination therapies can be used to promote precision therapy in an analogous manner to PARPi utilization in HR-deficient cancers.

These discoveries increase the existing armamentarium to rationally administer DNA-damaging agents in specific DNA-repair-deficient cancers. While parallels exist to PARPi in HR deficiency, there are notable differences. Irifolven was active in cells that contain a single NER-mutant allele, raising the possibility that it would be toxic in patients with the common germline NER variants. Additionally, irifolven directly creates DNA adducts rather than targeting an orthogonal mechanism that channels into the repair deficiency in question.

Collectively, these observations suggest a need to re-examine efforts that rely solely on genetic loss of function approaches to identify synthetic lethal interactions. Notably, PARPi is more toxic to HR-deficient cells than PARP1 deletion because inhibited PARP1 traps onto DNA(7). Moreover, PARPi is particularly effective in the setting of genomic lesions that trap the PARP enzymes on DNA, producing toxicity when the majority of cellular PARP activity is still present. These data support a hybrid model for PARPi action that combines loss-of-gene function together with DNA adduct formation (Figure).

Hence, the pharmacology of anti-cancer agents is typically not representative of purely synthetic lethal interactions. This is important to consider given the rarity of synthetic lethal pairs for most genes(3). Adapting base editing strategies to engineer enzymatically inactive missense alleles on a high throughput scale may offer tools that more closely recapitulate pharmacologic inhibition(6). Increased understanding of how genome structure creates unique DNA repair requirements also represents an untapped opportunity to identify targets, as recently reported for the Werner helicase dependency of cancer cells with microsatellite instability (10).

The findings from the Szallasi and Offit groups establish a new path to rejuvenate the development of irifolven. General cytotoxic therapy strategies have fallen out of favor in clinical development because of the higher bar achieved with more modern approaches. However, with improved understanding of DNA repair mechanisms and better molecular diagnostics, the potential therapeutic advance of selective DNA-damaging agents is newly illuminated. As new fundamental insights into the complex workings of genome maintenance are revealed, it might be morning again for candidate therapeutics like irifolven that had, for a time, been “put to rest.”

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Figure. Targeting DNA repair deficiencies in cancer.

Platinum chemotherapy causes intra- and inter-strand DNA crosslinks that are repaired by TC-NER and a combination of FA (Fanconi Anemia) and BRCA dependent HR mechanisms. Irofulven leads to DNA adducts that specifically require repair by TC-NER. ERCC2/3 encodes the DNA helicase XPD and XPB respectively, which are subunits of the TFIIH complex. Their mutations lead to deficient TC-NER and hypersensitivity to Irofulven. PARP inhibitors (PARPi) trap PARP1/2 on DNA, leading to increased repair by BRCA1 and BRCA2 dependent HR. HR deficient cells fail to repair DNA damage caused by PARPi.