Ezh2-dependent therapies in bladder cancer: synthetic lethality

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Bladder cancer (BC) is an epigenome disease

Tumor cells arise as a consequence of the acquisition of defined properties, due to gene alterations, which control the equilibrium of cell proliferation and survival. Most targeted therapeutic approaches are focused on the inhibition of those alterations leading to gain of function. This situation is different when these acquired properties are used to loss of function, which usually affect tumor suppressor genes. This accounts for a limited use of these alterations as therapeutic targets. However, this potential problem can be overcome by the use of synthetic lethality approaches (see below).

BC is a current clinical and social problem. This group of diseases display high incidence, prevalence and mortality rates (1). Moreover, the more indolent subtypes, the superficial BCs, display the highest incidence of disease relapse among cancer, which in some cases also arise with aggressive characteristics. This requires the continuous monitoring of patients, even at this relatively good prognostic stage (2). In addition, the therapeutic approaches for BC monitoring have not been improved for decades, thus making essential new therapeutic avenues. As in other tumor types, these can be based on the specific genomic alterations. Genomic approaches of BC understanding have revealed multiple similarities with other cancers. However, BC also display predominant alterations in chromatin remodelers, either by mutation or by their altered expression/function, thus causing histone modifications different from those observed in normal bladder urothelium (3). These epigenetic chromatin modifiers control reversible post-transcriptional changes such as acetylations or methylations, to express or repress genes. At least 89% chromatin and 64% nucleosome remodelers are altered in BC (4).

In view of the taxonomy of BC into different subtypes (5), the traditional classification of this disease, as non-muscle invasive (NMIBC) and muscle invasive tumors (MIBC), is now improved. Currently, the NMIBC are treated by transurethral resections while MIBC are treated with cystectomy, which in both cases can be followed by different adjuvant therapies. The characterization of genetic and epigenetic alterations can help to identify patients at high risk of recurrence and progression, and, remarkably may help to define more adequate therapeutic approximations. The aberrant epigenetic landscape is a hallmark of human cancer (6) and characterizes BC as an epigenome disease, opening new horizons to epigenetic therapy for the treatment of this disease.

Synthetic lethality as therapeutic approach

Synthetic lethality happens when the expression of two genes (mutated or not), but not each of them alone, promotes cell death. This lethal relationship can take place either by gene alterations or by inhibitory cytotoxic compounds acting on tumor cells. Additionally, as these characteristics do not exist in normal cells, this approximation could be of extreme benefit to cause tumor cell death without interfering with non-transformed cells in the organism. Thus, those alterations that identify tumor cells can convert into inducible synthetic lethality

therapeutic targets and provide a new possibility of personalized treatments based in the alterations of genes, whose products are considered undruggable such as RB1, Tp53, BRCA1, RAS and C-Myc (7,8).

Nonetheless, the existence of such lethal interactions should be studied in deep. There are several approaches mostly based on screening of large libraries of shRNAs (9), siRNAs (10), or CRISPR/Cas9 (11). However they differ not only in the technology used, but also on the possible different outcomes of the screening, as they are based on different temporal and distinct levels of gene inactivation/repression. Accordingly, these approaches may have different advantages or disadvantages regarding their use in the identification of potential synthetic lethality counterparts (9). With respect to targeted therapies directed against epigenetic alterations, synthetic lethality emerges as a very attractive approximation, decreasing unwanted side effects of chemotherapy and reducing the possibilities of drug resistance.

The alterations of histone demethylase KDM6A gene, either by silencing, often deep deletions or putative truncating mutation, occurs in 24% of bladder tumors (4), with even increased incidence in NMIBC (32–43%) (1,3). There is a rate of alteration similar in other epigenetic remodelers, such as ARID1A which is part of the SWI/SNF complex (6). These "loss of function" alterations have led to their assumption as putative tumor suppressor genes, and thus can help to identify synthetic lethality mechanisms.

The complexity of EZH2 as druggable target

EZH2 is a methyltransferase, which mediates gene silencing through the trimethylation of K27 of Histone H3. It is the catalytic subunit of the polycomb repressor complex II, together with factors EED and SUZ12. Increased expression of EZH2 may exert oncogenic functions, as it leads to gene repression, which can affect several tumor suppressors (12). This oncogenic capacity has also led to the design of potential EZH2 inhibitors, some of them are currently tested in preclinical and clinical trials (1,13).

KDM6A is a histone H3 Lys27 trimethyl (H3K27me3) demethylase and its function opposes that of EZH2. Whereas KDM6A displays alterations related with loss of function in many human tumors, EZH2 is frequently overexpressed correlating positively with high grades and worse prognosis (1,12).

The functional antagonism between these two enzymes has been recently utilized in preclinical BC therapy. Ler *et al.* reported that the loss of KDM6A promotes enrichment in PRC2-regulated signaling and confers specific vulnerability to EZH2 inhibition (11). Using different experimental approaches, they demonstrated that inhibition of EZH2 causes a delay in tumorigenesis and tumor progression in both BC cells after KDM6A inactivation and in patient-derived xenografts models. This represents an excellent example of synthetic lethality in cancer therapy context, and highlights the relevance of EZH2 as a putative target for BC management. However, there are aspects that still remained unsolved.

On the first instance, similar susceptibilities to EZH2 inhibitors have been characterized in other tumors in relation with mutations in ARID1A, ARID1B, PBRM1 or SMARCA4, known components of the SWI/SNF complexes of chromatin remodeling (7,10,14). However, these SWI/SNF mutants are primarily dependent on a non-catalytic role of EZH2 (7). Therefore is necessary to define whether inactivating mutations of these genes co-exist with those of KDM6A, and whether in those cases the activity of possible inhibitors depends on EZH2 histone methyltransferase activity. In this regard, since EZH2 may methylate non-histone substrates being independent of PRC2 (15), it is mandatory to determine whether other potential substrates are affected by these inhibitors.

On the other hand, despite the existence of several inhibitors of EZH2, these are still in the preclinical or early clinical development. Moreover, various manners of inhibiting EZH2 functions have been proposed, such as SAM-hydrolase or SAM-competitive inhibitor compounds, inhibitors of the PRC2 complexes stability, etc. (16). In addition, the loss of EZH2 function can be compensated by its EZH1 counterpart, which its essential roles in the progression and maintenance of some myeloid neoplasms have recently been demonstrated (17), indicating that the combination therapy directed at these two enzymes could be more effective antitumor than a selective EZH2 inhibitor (17-19). Thus more exhaustive pharmacological studies are extremely needed.

Another possible point of complexity might come from the existence of mutations in EZH2. Although these are extremely rare in BC, they are common in other cancer types, such as hematologic tumors producing a gain of function (20) or inactivation (21,22). Moreover, prolonged depletion of EZH2 may produce altered tumor cell identity and tumor progression (23), indicating a precise drug dosing regimens. As a consequence the use of EZH2 inhibitors in the clinic must be considered cautiously to avoid unwanted

secondary side effects.

Another problematic issue could be due to the acquisition of resistance. In general, this can be produced by novel gene alterations leading to the activation of different signaling pathways to maintain cellular viability (9,13). Therefore, the emergence of EZH2 inhibitors resistance events should be carefully analyzed. In this context, experimentally induced resistance models of lymphoma revealed the requirement of inhibiting wild type and mutant forms of EZH2 (13). Also, novel secondary EZH2 mutations may confer resistance to EZH2 inhibitors (1), whereas loss of PRC2 subunits promoted the amplification of genetic signatures associated to Ras oncogene activation (1).

Collectively, new alternatives for the management of BC cancer patients emerged from these ongoing initiatives, using the specific sensibility attributable to precise gene mutations, in particular, exploiting the epigenetic remodeler alterations. Nonetheless, a better understanding of their subjacent mechanistic roles and new pharmacological development of accurate inhibitors are strictly required.

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Footnote

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