MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 6, e1809959 (3 pages)
https://doi.org/10.1080/23723556.2020.1809959

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AUTHOR'S VIEWS

Tumorigenic de-differentiation: the alternative splicing way

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ABSTRACT

The mechanism of acquisition of tumorigenic properties by somatic cells at the onset of cancer and later during relapse is a question of paramount importance in cancer biology. We have recently discovered a Muscleblind like-1 (MBNL1)-driven alternative-splicing mediated mechanism of tumorigenic de-differentiation that is associated with poor prognosis, relapse and metastasis in common cancer types.

ARTICLE HISTORY
Received 3 August 2020
Revised 7 August 2020
Accepted 10 August 2020

KEYWORDS
MBNL1; MAP2K7; JNK; JNK inhibitors; alternative splicing; cancer cell de-differentiation; cancer stem cells; RNA binding proteins; splice factors

Muscleblind-like-1 (MBNL1) is an RNA binding protein that functions as a master-regulator of RNA-processing. It regulates alternative splicing,[1,2] alternative polyadenylation,[3] transcript localization[4] and transcript stability.[5,6] MBNL1 stabilizes or destabilizes key transcripts involved in metastasis to suppress metastasis in breast and colorectal cancers.[5,6] In other solid tumors, MBNL1 regulates tumor-associated alternative-splicing networks and behaves as a tumor suppressor.[1] Recently MBNL1 was shown to be an oncogene in Mixed-lineage leukemia (MLL)-rearranged leukemia where its over-expression regulates the alternative splicing of key transcripts such as *DOTL1* and *SETD1A* that drives leukemia.[7] Furthermore, an exon7 included isoform of MBNL1 was found to occur recurrently in prostate cancers and function as a dominant negative.[8] It is evident, therefore, that MBNL1 is emerging as an important tumor-driver that is perturbed at both the transcriptional and post-transcriptional (splicing) level in multiple cancer types. A comprehensive understanding of MBNL1 function and its mechanism of action is of prime importance to the field.

In our recent work, we discovered that *MBNL1* expression is significantly reduced across several common cancers. Low *MNBL1* expression is associated with poor prognosis, relapse and metastasis in many common cancer types. Low MBNL1 expression causes transcriptomic alterations akin to what is reported for stem-like cellular state, both in gene-level expression as well as in alternative-splicing of transcripts. We showed that tumor cells expressing low levels of MBNL1 behave similarly to stem-like cells. In RNA-seq data from breast and stomach cancer patients from the cancer genome atlas (TCGA), low *MBNL1* expression levels correlated to increased expression of stemness gene signatures (Figure 1).[2] It is hypothesized that a cancer stem-like cell may arise either by malignant transformation of normal tissue-specific stem/progenitor cells or via re-programming of mature somatic cells. Our data highlights a role of MBNL1 in tumorigenic de-differentiation of cells by re-wiring alternative splicing.[2] It is important to note that stem-like features of cancer cells are central to tumor heterogeneity, cancer initiation, metastasis and relapse.

Transcriptomic studies have established cancer-associated splicing as a common feature of cancer.[1,9] However, how individual splicing alterations impact protein function in order to contribute to tumorigenesis is largely understudied. In our work, we discovered that a discrete set of 12 'Embryonic stem cell (ESC)-differential' splice isoforms (described in[10]) are upregulated in MBNL1-low cancers. Each of these 12 'cancer stemness associated' splice isoforms has interesting biology that ought to be investigated for the complete understanding of the role of splice isoforms in driving tumor cell de-differentiation. In our recent work, we focused on the exon2 skipped isoform of *MAP2K7* (referred hereafter as *MAP2K7*Δexon2), which is shared between many MBNL1-low cancer types. MAP2K7 (also known as MKK7) is a mitogen-activated kinase protein that activates c-JUN N-terminal Kinase (JNK) signaling by directly phosphorylating JNK 1, 2 and 3. The skipping of exon2 of *MAP2K7* is known to form a high-affinity JNK docking site leading to activation of JNK signaling.[11] We discovered that the *MAP2K7*Δexon2 splice isoform is upregulated in many *MBNL1*-low cancers and that this isoform largely drives the MBNL1-downregulation mediated tumor de-differentiation via activation of a JNK signaling mediated feedback loop. We further discovered that this MBNL1-*MAP2K7*Δexon2 mediated tumor de-differentiation is reversible by JNK inhibition (Figure 1).[2]

JNK is a well-known therapeutic target in oncology, particularly as a means to specifically target cancer-stem-like cells.[12-14] However, so far small molecule inhibitors of JNK have not succeeded in clinical trials due to toxic side effects.

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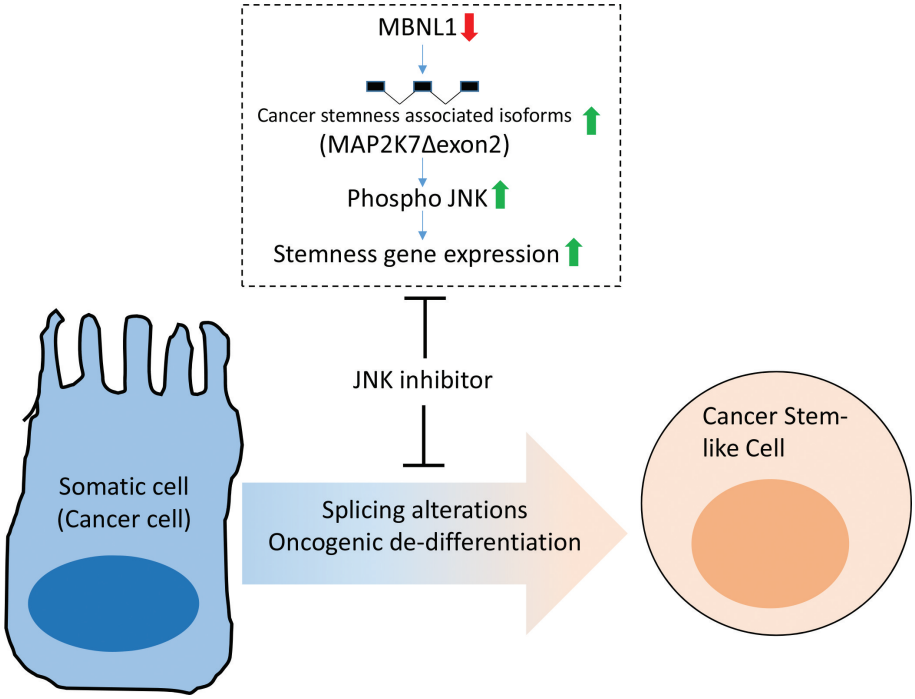

Figure 1. Mechanisms whereby alternative splicing causes the de-differentiation of somatic cells to cancer stem-like cells.
Schematic diagram showing de-differentiation of somatic cells to cancer stem-like cell by downregulation of MBNL1 (Muscleblind-like-1) leading to the upregulation of cancer stemness associated splice isoform *MAP2K7*Δexon2 (exon2 skipped *MAP2K7* isoform) and consequent JNK (c-JUN N-terminal Kinase) activation. This mechanism of tumorigenic de-differentiation is reversible by inhibition of JNK signaling.

JNK has a multitude of essential cellular functions and the role of JNK signaling in cancer is highly context-dependent, making clinical targeting challenging.[15] We reveal a molecular context for JNK activation in cancer, i.e. presence of *MAP2K7*Δexon2 isoform in *MBNL1*-low cancers with high stem/progenitor-like properties (Figure 1).[2] Our findings have important clinical implications. Biopsies from cancer patients can be assayed for *MBNL*1 and *MAP2K7*Δexon2 isoform expression as patients with low *MBNL1* and high *MAP2K7*Δexon2 would be likely to benefit from JNK inhibition. These biomarkers predict not only increased stem/progenitor-like properties but also enhanced susceptibility to JNK inhibition. Currently, promising JNK inhibitors like D-JNKI-1 are in clinical development for hearing loss and ocular inflammation.

Our work highlights the role of MBNL1, a regulator of RNA processing, in regulating tumorigenic de-differentiation via upregulation of cancer stemness-specific splice isoform. This data places aberrant alternative-splicing alongside dysregulation of gene-expression as an important regulator of cancer cell plasticity. It highlights how change in protein function arising as a result of alternative-splicing may affect cellular signaling that contributes to tumorigenic properties. Most importantly our data provide a molecular context of JNK activation in cancer cells with stem/progenitor-like properties that may be useful for patient prognostication for any future anti-JNK therapy. Our work may also lead to novel and alternative ways of targeting the JNK pathway that may circumvent toxic effects caused by conventional small-molecule mediated JNK inhibition. It also throws open interesting questions regarding the mechanism of MBNL1 transcriptional dysregulation in cancer. Analysis of publicly available cancer genomics data makes it clear that MBNL1 locus does not get affected by recurrent mutations or copy number variations. We also did not observe promoter methylations of MBNL1 in stomach cancer cell lines indicating that MBNL1 downregulation might be triggered by other epigenetic mechanisms. Also, interestingly we observed a renal cancer-specific upregulation of MBNL1. This indicates a renal-specific function for MBNL1 which may be similar to the MLL-rearranged leukemia. In conclusion, MBNL1 and MBNL1 processed transcripts (spliced, stabilized or spatially localized) are emerging as novel and key players in cancer and we are yet to unravel the full impact of MBNL1 perturbation in cancer.

Disclosure of potential conflicts of interest

D.R. is an inventor on a provisional patent that uses MAP2K7 and MBNL1 for prognostication of cancer patients for anti-JNK therapy. D.M.E. is the founder director of Black Diamond Therapeutics and consultant for Engine Biosciences in areas unrelated to this manuscript.

Funding

This work was supported by the National Medical Research Council [NMRC/OFYIRG/0065/2017].

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