Published OnlineFirst June 1, 2015; DOI: 10.1158/1078-0432.CCR-15-0883

CCR Translations

Clinical Cancer Research

SLFN11: Achilles' Heel or Troublemaker

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SLFN11 expression correlates with sensitivity of tumors to topoisomerase and DNA-targeting drugs and consequently with prognosis. Regulation of SLFN11 by ETS factors opens new avenues to treatment optimization, maximizing antitumor activity and minimizing adverse side effects. Interrogating drug-induced gene expression signatures for SLFN11 modulations may affect the design of therapeutic regimens. *Clin Cancer Res; 21(18); 4033–4. ©2015 AACR.*

See related article by Tang et al., p. 4184

In this issue of *Clinical Cancer Research*, Tang and colleagues (1) report on the regulation of *SLFN11* by ETS transcription factors, specifically by the chimeric oncoprotein EWS–FLI1 in Ewing sarcoma. The Schlafen (*SLFN*) family of genes, restricted to mammals, encodes for proteins of still enigmatic function. Based on motif similarity with nucleic acid sensors RIG-1 and MDA-52, SLFN11 is assumed to have DNA/RNA helicase activity. Interferon inducibility and amply documented sensitization of cancer cells to topoisomerase inhibitors suggest a function in cellular stress response, in particular to DNA damage. By a hypothesis-driven approach and in line with a study of tumor xenografts from the Reynolds' laboratory recently published in *Clinical Cancer Research* (2), Tang and colleagues confirm this observation in Ewing sarcoma and demonstrate in a small cohort of 44 patients that SLFN11 RNA expression might be of prognostic power. If confirmed in multivariate analyses of larger independent cohorts, this finding may aid in treatment stratification of low-risk and high-risk Ewing sarcoma patients. The potential inclusion as a prognostic marker into the routine diagnostic workup of tumor samples would profit from further developing SLFN11 detection by immunohistochemistry.

The authors' major novel finding is that SLFN11 expression is subject to regulation by ETS oncogenes. They identify RNA expression of a number of different *ETS* genes to correlate with SLFN11 expression in the NCI-60 cancer cell line panel and in The Cancer Genome Atlas (TCGA) dataset, on top of them FLI1. This finding, originally based on EWS–FLI1 chromatin binding data in a Ewing sarcoma cell line (3), is supported by mutational analysis of two ETS consensus binding motifs in *SLFN11* promoter activity assays. As exemplarily demonstrated for EWS–FLI1 and ETS1, the *SLFN11* promoter is highly promiscuous for ETS factor binding and activation, and the presented gene expression correlation analysis suggests that various different ETS factors may activate *SLFN11* in a cell type–specific manner. A recent Ewing sarcoma epigenome study showed that EWS–FLI1 binding to promoters, in contrast to enhancers, is primarily associated with broadly active and widely expressed genes, where it acts predominantly as an amplifier of preexisting gene expression (4). In fact, in two model systems, human umbilical cord vascular endothelial cells and mouse embryonic superficial zone cells, ectopic expression of EWS–FLI1 led to the replacement of endogenous FLI1, respectively ERG, at ETS-driven promoters (5, 6), whereas knockdown of EWS–FLI1 resulted in its replacement from target promoters by ELF1 and GABPA ETS family members in a Ewing sarcoma cell line (7). Taken together, it is likely that pathogenic activation of certain ETS family members such as of FLI1 or ERG in Ewing sarcoma boosts preexisting SLFN11 expression, consistent with the results of the current study.

This is good news given the established sensitization effect of high SLFN11 expression to DNA-targeted chemotherapy. On the other hand, it rings an alarm bell: FLI1, ERG, and other ETS family members are particularly highly expressed in tissues affected by treatment-induced toxicity of these very same drugs. ERG and FLI1 have been shown to be required for endothelial differentiation and lineage specification, in particular in the cardiovascular and hematopoietic system (8–11). ETS-2 and ERG modulate cardiac matrix metalloproteases and affect cardiac structural development and remodeling. ETS domain proteins, the ternary complex factors ELK1, ELK3, and ELK4, influence T-cell–positive selections via serum response factors. ETS factors also mediate transcriptional effects upstream and downstream of nitric oxide, a signaling molecule generated in response to anthracycline treatment that is, at least in part, responsible for anthracycline-mediated cardiotoxicity. Therefore, it is of interest if SLFN11 expression also correlates with these ETS factors in normal tissues, and, if it does, whether this may explain some of the most frequently observed adverse side effects of DNA-targeted compounds in cancer patients. As a first clue to this question, we looked into the "Illumina RNA-Seq Bodymap" (Array Express Accession: E-MTAB-513) and confirmed a high correlation ($R = 0.79$) between FLI1 and SLFN11 expression in normal tissues too (M. Kauer; unpublished observations). It is possible that even if SLFN11 expression in healthy tissues similarly associates with sensitivity to DNA-damaging drugs, the slope of sensitivity may be less steep than in tumors because of multiple additional aberrations in stress response genes. Ewing sarcoma, for example, overexpresses not only SLFN11 but also PARP1, and Tang and colleagues here demonstrate that this constellation renders Ewing sarcoma cell

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doi: 10.1158/1078-0432.CCR-15-0883

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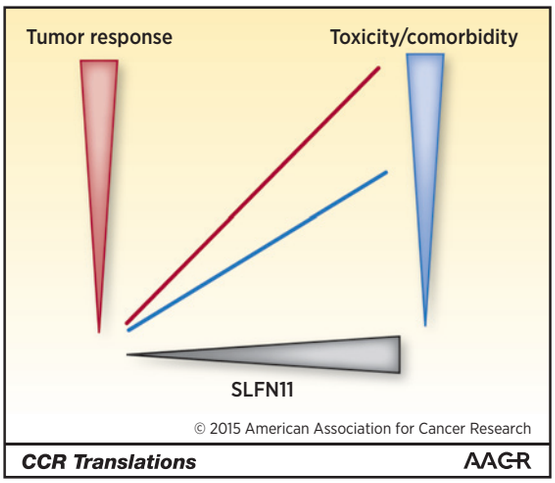

Figure 1.
SLFN11 expression levels are expected to correlate not only with tumor drug response but also with toxicity and morbidity of normal tissues (cardiovascular and hematopoietic). Different slopes may open a window of opportunity for SLFN11 expression–guided drug-dosing optimization.

lines particularly sensitive to combination treatment of alkylating temozolomide with the PARP1 inhibitor niraparib. The potential therapeutic implication of Tang and colleagues' findings in this context would be that patients with high tumor SLFN11 expression, due to their higher sensitivity to topoisomerase inhibitors and to other DNA-targeting drugs, might profit from a dose reduction to minimize damage to comorbid normal tissues while still maximally hitting the tumor. While such a concept may be applicable to a wide variety of cancers, the pediatric population would profit the most. Here, the guiding dosing principle is "as much as absolutely necessary but not more than that," to reduce late effects and prolong life span (Fig. 1).

Tang and colleagues also identified a further potential therapeutic problem ensuing from their results: If EWS–FLI1 drives expression of a chemosensitizer, for example SLFN11, any treatment with an EWS–FLI1-targeting compound would be expected to support chemoresistance. This conclusion may still be a bit premature, because, similarly to PARP1, the role of SLFN11 in DNA-damage response and the mechanism how it affects response to DNA-targeting compounds remains unknown. Expression signature-based approaches have been used to identify EWS–FLI1-targeting compounds. Surprisingly, several widely used anticancer drugs, including the topoisomerase inhibitor etoposide, a backbone component of almost all Ewing sarcoma therapies, were demonstrated to result in EWS–FLI1 attenuation-like gene expression changes (reviewed in ref. 12). It will therefore be of interest to revisit drug-induced expression signatures to interrogate response of SLFN11 expression in these datasets. If already induction chemotherapy reduces SLFN11 expression in surviving tumor cells, this may in fact have adverse implications for treatment success. At this point, therefore, it remains open if regulation of *SLFN11* by ETS factors is good or bad news for the clinical management of Ewing sarcoma and other ETS-driven cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

H. Kovar was supported by the EU-FP7 grant 259348 "ASSET."

Received April 16, 2015; accepted April 17, 2015; published OnlineFirst June 1, 2015.

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Published OnlineFirst June 1, 2015; DOI: 10.1158/1078-0432.CCR-15-0883

AACR American Association for Cancer Research

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Clin Cancer Res 2015;21:4033-4034. Published OnlineFirst June 1, 2015.