CANCER

Beyond static biomarkers—The dynamic response potential of signaling networks as an alternate biomarker?

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In this week's issue of *Science Signaling*, Fey *et al.* introduce a new type of biomarker. Using the example of neuroblastoma, the authors demonstrate that patient-specific differences in the computed property (the Hill coefficient) of the dynamics of a pathway involved in cell death signaling outperformed the prognostic capability of any single static biomarker alone or in combination.

Traditional cancer biomarkers are based on the association between the abundance of a specific protein or transcript (or the presence of specific mutations in the tumor) and clinical outcome. However, the use of multiparametric gene signatures as a standard tool for clinical diagnosis faces major impediments, such as the typically low reproducibility of these signatures and the difficulty of achieving a clear biological interpretation (*1*). In *Science Signaling*, Fey *et al*. introduce a new type of biomarker based on the dynamic properties of a signaling pathway and provide proof of concept using neuroblastoma patient data (*2*).

Neuroblastoma is the most common extracranial solid tumor in children and affects about 8% of the pediatric population. The clinical presentation is quite variable, ranging from spontaneous regression to life-threatening disease. However, there is no validated biomarker today that would enable physicians to distinguish the patients who would benefit from an aggressive treatment from those who merely require careful watching (*3*).

Intense efforts have focused on genome-wide association studies (GWAS) in neuroblastoma, as well as whole-exome and whole-genome sequencing studies with the goal of identifying driver mutations. However, similar to other pediatric tumors, the number of associated mutations in neuroblastoma is quite low (*4*). At present, *MYCN* amplification remains the best-characterized genetic marker of risk in neuroblastoma, despite efforts to identify epigenetic or expression-based biomarkers. *MYCN* amplification is found in ~25% of cases and correlates with high-risk disease (*5*). However, many patients lacking *MYCN* amplification also have a poor prognosis, and there is little understanding of the biochemical pathways that drive tumorigenesis and chemoresistance in these patients (*6*).

Fey *et al*. propose a novel biomarker approach to facilitate the prognostic staging of neuroblastoma and to ultimately support physicians in their treatment decisions (*2*). The authors' key hypothesis is that signaling dynamics are key drivers of cell decisions and therefore should contain critical prognostic information. They examined three properties—signal amplitude, Hill coefficient (a measure of ultrasensitivity in the pathway), and half-activation threshold—computed from pathway dynamics using information about the abundance of relevant transcripts for components in the pathway from individual patients. Computing the Hill coefficient that describes the strength of Jun N-terminal kinase (JNK) activation (i.e., phosphorylation) in response to a stress stimulus for individual patients could identify patients with poor prognosis (= low Hill coefficient) (Fig. 1). Furthermore, the Hill coefficient, but not signal amplitude or half-activation threshold, correlated best with patient prognosis in neuroblastoma.

Mechanistic models have become a commonly used tool to understand how signals are transduced in cells and how cell fate decisions are made (*7*). Computational models have been used previously to identify drug targets and design novel therapies (*8*); however, this is the first time that an "in silico"–derived biomarker capturing the nonlinear dynamics of a key signal transduction pathway for a particular disease outperformed the predictive capabilities of a single marker to accurately stage patients.

Overwhelming evidence suggests that the JNKs are a set of key stress-responsive kinases that mediate apoptosis, which is an important process for tumor suppression. However, JNKs have also been implicated in the malignant transformation and tumorigenesis of cells (*9*). Fey *et al*. demonstrated that suppression of the JNK signaling pathway is critical for tumor progression and chemoresistance in neuroblastoma and that JNK activation plays a central role in the apoptotic response of neuroblastoma cells to a wide variety of stimuli (*2*). On the basis of the preclinical work, Fey *et al*. hypothesized that a high-amplitude, ultrasensitive JNK response would promote apoptosis in neuroblastoma lines and therefore correlate with improved prognosis in patients whose tumor cells are in silico capable of an ultrasensitive JNK activation response to stress (*2*).

Initially, the authors curated and experimentally characterized the stress-induced JNK signaling pathway in neuroblastoma cell lines to build a computational model, using a rule-based modeling approach that described the biochemical reaction network of the JNK signaling pathway. The model was calibrated and validated using SH-SY5Y cells and anisomycin as the stressor input. Subsequently, they used the model to predict the JNK activation response to different stressors in multiple neuroblastoma cell lines. The authors measured the amount of each kinase in the model for each cell line and populated the original SH-SY5Y model with these values as parameters, thereby generating cell line–specific models. Simulations predicted that the activation of JNK would significantly vary from a high-amplitude ultrasensitive response to a suppressed, flat response across the cell lines; experiments confirmed these predicted differences in stress-activated JNK activation.

Turning to neuroblastoma patient data, they used a cohort of 109 patient samples as a training data set, and two additional patient data sets (369 patients and 233 patients) were used as independent validation cohorts. For each patient, the protein concentrations in the model were adjusted according to the relative amount of the encoding transcript measured in the tumor. To compare the capability of each patient to induce an ultrasensitive JNK response, three dynamic network descriptors were assessed: the maximal amplitude, the activation threshold, and the Hill exponent. The optimal cutoff values for each dynamic network descriptor were determined using Kaplan-Meier scanning. The minimum *P* value of the overall survival difference in the training data set identified the network descriptor cutoff value that was later applied to the validation patient data sets. The Hill coefficient, which is a measure of ultrasensitivity of the JNK activation response, pro-

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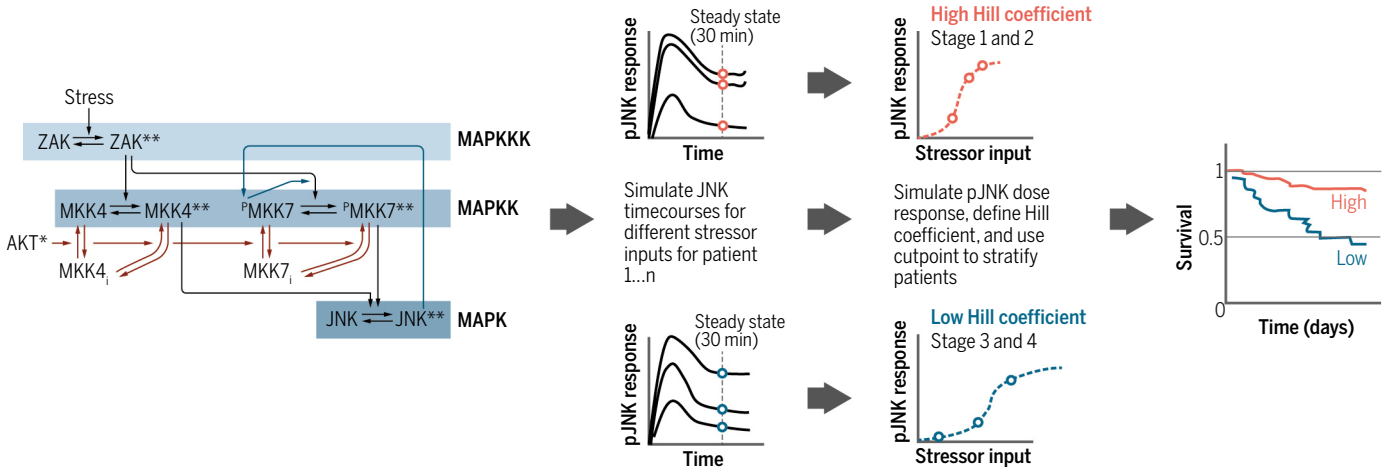

Fig. 1. Using network descriptors of signaling pathway activation potential to predict patient response. After construction of a computational model based on the validated network topology and that reproduces the signaling pathway dynamics, the model can be used to identify network descriptors, such as the Hill coefficient, that are calculated from the dynamic simulation of the activation of a signaling pathway. These in silico biomarkers cannot be directly measured.

vided the most accurate patient stratification, using the same cutoff value in the validation cohorts and the training cohort. Although *MYCN* amplification status is associated with an impaired JNK activation response in neuroblastoma, the authors clearly showed the limitations of only using *MYCN* amplification status or the individual nodes of the JNK signaling network as predictive markers, relative to using the Hill coefficient. Even after the *MYCN* amplification status was used to group patients, the simulated dynamic network descriptors provided additional information on the relationship between JNK response and patient prognosis. Furthermore, the dynamic descriptors consistently showed better predictions on patient outcomes when compared to each signaling node, which underscores the importance of network topology and nonlinear system dynamics in disease. The effects of these dynamic properties cannot be captured if only the baseline expression levels or abundance of individual network components are used as biomarkers. Because the JNK signaling pathway is highly nonlinear as a result of feedback regulation and scaffolding, it would be difficult to delineate nonlinear biological responses with multivariate analysis, such as partial least-squares regression or artificial neural network models. In addition, unlike regression-based methods, the mechanistic model provides causality for the correlative relationship found from the analysis.

The work by Frey *et al*. shows that not only the levels of signaling nodes but also the network topology and thus the nonlinear properties of a signal response should ideally be captured in a biomarker to robustly predict patient outcome. This can only be accomplished in the context of a mechanistic computational model. The in silico–derived network descriptor, the Hill coefficient, outperformed the single protein–based or gene expression–based biomarkers alone or in combination. Given the contradictory reports about the role of JNK signaling in other tumor types, it would be interesting to see whether the Hill coefficient of JNK activation is also prognostic in other cancers (*10*, *11*). From a practical perspective, the translation of such an "in silico" biomarker into a commercial diagnostic test will be challenging, because it will require validated diagnostic tests of multiple markers as well as a fully validated computational model.

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