How to Train Your Biomarker

Douglas Yee

Type I insulin-like growth factor (IGF) receptor (IGF1R) inhibitors are new cancer therapies. Pitts and colleagues used in vitro data to “train” a predictive biomarker for an IGF1R tyrosine kinase inhibitor. Given the complexity of IGF signaling, additional layers of biomarker analysis will likely be needed to develop predictive factors. Clin Cancer Res; 16(12); 3091–3. ©2010 AACR.

In this issue of Clinical Cancer Research, Pitts and colleagues develop biomarkers to predict sensitivity to a type I insulin-like growth factor receptor (IGF1R) tyrosine kinase inhibitor (TKI; ref. 1). Numerous anti-IGF1R drugs are in clinical trial, based on the strength of population and preclinical data showing the role of this receptor system in the malignant phenotype. This transmembrane tyrosine kinase receptor functions as a covalently linked homodimer and is highly homologous to the insulin receptor (InsR). Indeed, hybrid receptors between IGF1R and InsR exist and function to elicit “IGF-like” responses. To date, a robust predictive biomarker has not been developed.

Their study examines response to the IGF1R inhibitor OSI-906. This TKI disrupts both IGF1R and InsR signaling at approximately equimolar concentrations. Using protein expression, gene expression profiling, and mutational analysis, Pitts and colleagues discovered that sensitivity in colorectal cell lines can be determined by examining expression of pathway molecules. Expression of the IGF1R pathway components or by using the "k-TSP" (k-top scoring pairs) classifier with additional information on KRAS status and IGF1R copy number, determined by fluorescence in situ hybridization (FISH), predicted sensitivity in xenograft tumors. Importantly, Pitts and colleagues show that measurement of IGF1R alone is not informative; a more detailed analysis of the signaling pathway can distinguish drug sensitivity. Thus, "training" a biomarker in vitro yields a potential clinically useful tool.

But is this enough? What is the best way to train a laboratory biomarker for use in the clinic? It must be acknowledged that the IGF1R and the InsR function in an endocrine signaling system. Host receptor perturbation will likely have therapeutic implications. There are potentially several layers of IGF effectors (Fig. 1) that could serve as biomarkers.

Ligands

Three ligands (IGF-I, IGF-II, and insulin) can activate the IGF1R, hybrid receptor, and insulin receptor. Indeed, the receptors are physically constrained by covalent bonding making it very difficult to initiate signaling in the absence of ligand. Even when the receptors are over-expressed in model systems, ligand activation is required for function (2). Population studies also show the importance of ligand; elevated IGF-I levels are associated with cancer risk (3). Early reports suggest that elevated ligand levels might predict response to anti-IGF1R antibodies (4). Importantly, disruption of IGF1R with monoclonal antibodies results in elevations of growth hormone, IGF-I, and insulin in humans (5), which could serve as a potential resistance pathway by activating residual InsR (see below).

Receptors

At least four receptors can bind the IGF ligands: holo-IGF1R, holo-InsR, hybrid IGF1R/InsR, and the type II IGF receptor (IGF2R). The latter receptor sequesters only IGF-II and does not activate signaling. Although Pitts and colleagues find that levels of protein expression do not predict sensitivity to OSI-906, they did not evaluate receptor confirmation or the InsR. Because OSI-906 inhibits InsR, levels of this receptor could be a predictor of benefit. Moreover, the receptor confirmation (holo versus hybrid) could have profound implications for monoclonal antibodies directed against IGF1R. Because none of the developed antibodies bind InsR, a cell expressing mostly holo-InsR will not be affected by anti-IGF1R antibodies. In contrast, antibodies disrupt hybrid receptor signaling by binding IGF1R. A cell with all of the InsR chains involved in InsR/IGF1R hybrids might be exquisitely sensitive to blocking the IGF1R portion of the receptor. The IGF1R TKIs have little selectivity for either IGF1R or InsR, but there is still a need to measure both receptors and their conformation before it can be determined that receptor levels do not predict response. Indeed, Pitts and colleagues...
Once downstream of the adaptor proteins, numerous pathways are activated by IGF1R and InsR, yet it is difficult to ascribe sensitivity to any single kinase receptor. For example, it is clear that phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin complex 1 (mTORC1) is a critical pathway in IGF signaling, yet many growth factor receptors use this pathway. Specificity could be shown by examining samples that are studied before and after drug exposure. These samples will be captured in the I-SPY2 neoadjuvant trial in breast cancer (8). Tumor biopsies pre- and post-anti-IGF1R treatment will be studied to measure dynamic signaling interactions.

**Gene Expression Profiling**

The signaling pathways initiated by IGF1R and InsR are complex and numerous. Ideally, it would be best to have a status of the phosphorylated proteins and lipids before and after any signaling intervention, but these types of assays are still under development. In contrast, gene expression profiling is a reasonably mature technology, and Pitts and colleagues use this platform to identify gene predictors. Creighton and colleagues have also created an "IGF-activated" signature, but have done so with a different experimental design (9). Although Pitts and colleagues evaluated the effects of OSI-906 in full serum, Creighton stimulated cells with and without IGF-I. Thus, they created a "before and after" gene expression profile. Importantly, inhibition of cancer cells with a TKI reverses this signature (10). Tumor-associated fibroblasts and stromal cells will also respond to IGFs. Rajski and colleagues showed that stromal fibroblasts have a distinct IGF-activated signature that may be different than the malignant epithelial cells (11).

Inhibition of IGF1R is a promising new therapy, but currently it is at an important crossroads. Although single agent activity for the anti-IGF1R receptor antibody figitumumab has been documented (12), promising preliminary data (13) for this drug in combination with cytotoxic chemotherapy in non-small cell lung cancer could not be reproduced in a larger phase III trial. Substantial questions about targeting this pathway remain: What is the role of the insulin receptor? Are the pathways regulated by this system easily validated in clinical trials? How can this strategy be combined with new and existing therapies? What biomarkers predict sensitivity? To address this last question, Pitt colleagues have shown how to train a biomarker that could be used in clinical trials.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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