In this issue of *Clinical Cancer Research*, Naik and colleagues (1) provide the rationale for a synthetic lethal therapy theoretically based on targeted inhibition of vascular endothelial growth factor receptor-1 (VEGFR1)-mediated kinase activity in Wnt/β-catenin-addicted human colorectal cancer (CRC) cells (Fig. 1). Wnt is a cysteine-rich secreted molecule and prototype of a 19-member family of proteins. It activates target genes primarily through binding and stabilization of the Frizzled/LRP6 membrane receptor complex. This complex sequesters cytoplasmic Axin thus preventing it from mediating the ubiquitination and degradation of β-catenin. Stable β-catenin is phosphorylated and translocated to the nucleus, where it targets the TCF/LEF family of transcription factors and promotes expression of genes that regulate cell proliferation and cell polarity (2). A significant proportion of CRCs have an activating mutation in the Wnt/β-catenin pathway resulting in the abnormal expression of proliferation mediators and growth factors (e.g., C-myc and VEGF; ref. 3).

Naik and colleagues (1) expose a direct link between VEGFR1 function and the Wnt/β-catenin signaling pathway in CRC cells. To determine this linkage, they developed a Wnt/β-catenin screening protocol conceptually based on the phenomenon of synthetic lethality (4). The authors used a series of small inhibitory RNA (siRNA) and short hairpin RNA (shRNA) panels to investigate a human embryonic kidney cell line (STF293) bearing a Wnt/β-catenin-responsive reporter gene. RNA interference protocols have been introduced recently as synthetic lethal screens and have already begun to uncover other unexpected linkages between genes and survival pathways that show promise for therapeutic intervention (4, 5). Notably, this technique uncovers functional weaknesses of mutations specifically mediating survival of cancer cells, thus allowing highly specific pharmacological targeting strategies.

The discoveries made by Naik and colleagues (1) shed new light on the complexities of VEGF-receptor signaling and function. VEGF binds to both VEGFR1 and VEGFR2, the primary VEGF receptors involved with angiogenesis and vasculogenesis. VEGFR1 has weak tyrosine kinase activity compared with VEGFR2. Interestingly, VEGF binds to VEGFR2 with weaker affinity than to VEGFR1, but it results in far stronger VEGFR2 tyrosine kinase activity (6). The biologic relevance of the unique and reciprocal characteristics of these two receptors remains to be fully understood. We have recently reported that VEGF-dependent tumor angiogenesis involves the inverse and reciprocal regulation of VEGFR1 and VEGFR2 (7). VEGFR2 undergoes endocytosis, nuclear translocation, and down-regulation via ubiquitination in response to VEGF-induced signaling through the JNK/c-Jun pathway. In contrast, VEGF/VEGFR1 signals through the Akt/ERK pathway to inhibit constitutive ubiquitination and induce rapid VEGFR1 protein accumulation in endothelial cells. Surprisingly, VEGFR1 is primarily localized intracellularly in endothelial cells (7, 8). VEGFR1 is strongly expressed in CRC cells, where it also shows an intracellular localization (9). In light of the current observations by Naik and colleagues on the importance of VEGFR1 for the survival of CRC cells, these results bring to light the challenges in development of therapeutic agents targeting VEGFR1, particularly antibodies designed to inhibit VEGFR1 function.

Naik and colleagues (1) identify VEGFR1, among others, as a synthetic lethal partner to the Wnt-signaling pathway. In confirmation of the screen data, VEGF tyrosine kinase inhibitors were effective at inhibiting growth of Wnt/β-catenin-addicted CRC cells, but not control cells bearing normal Wnt function. Does this new observation fit in with any currently recognized VEGF receptor-related mechanism? VEGFR2 is primarily expressed on endothelial cells, whereas VEGFR1 has been detected on

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6 L.M. Ellis, unpublished results.
However, off-target effects have been frequently observed with small molecule tyrosine kinase inhibitors, which hinders one's ability to clearly understand their exact mechanisms of action.

For high specificity, we may ultimately seek gene-silencing methodologies for effective and precise inhibition of VEGFR-1 pathway function. Some questions do exist about off-target effects of RNA-based gene-silencing methodologies, although providentially these effects may be anti-angiogenic in their own right (12). The stringency of nucleotide complementarity is high, however, tissue-specific delivery is inherently absent in these molecules. Fortunately, RNA interference (RNAi) is compatible with a number of modifiable clinically relevant and specific delivery systems, in particular viral packaging and liposome encapsulation. Notably, it is conceptually attractive to be able to identify intimately reliant tumor cell survival pathways using the same technology and mechanism, in this instance RNAi, which can be directly translated in vivo and potentially into clinical settings.

Everything being equal, inhibition of VEGF pathways is expected to attenuate angiogenesis and impair tumor growth. However, clinical experience is showing that this is frequently not the case. In light of the data described by Naik and colleagues (1), rational treatment with anti-VEGFR-1 antibodies may require the detailed study of cellular localization of the target receptors, as several receptors that are targets for therapy have been found to have intracellular functions independent of their kinase activities (13). Naik and colleagues (1) perhaps provide one additional explanation for the observed variable effects of anti-VEGF therapy, by linking the effect of VEGFR1 inhibition to the presence and/or absence of aberrant Wnt/β-catenin signaling. These exciting new observations reveal a new facet for selectivity of antitumor agents masquerading as anti-angiogenic agents, and underline the urgent need for deeper understanding of the biology of VEGF receptor signaling.

**Disclosure of Potential Conflicts of Interest**

L. Ellis, consultant, Schering-Plough; commercial research grant, Sanofi-Aventis, Imclone; advisory board, Genentech/Roche, Amgen, AstraZeneca.
References


Inhibition of Vascular Endothelial Growth Factor Receptor-1/Wnt/β-catenin Crosstalk Leads to Tumor Cell Death

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