We congratulate Adjei (1) on his excellent, comprehensive, and timely review on Ras signaling and the therapeutic implications of blocking this signaling. Perhaps space constraints and a major focus on pharmacologic aspects prevented any discussion of a promising alternative approach to perturbing Ras membrane localization, namely, the diversion of intracellular trafficking by an intracellular antibody (Fig. 1).

After the initial formal proof of intracellular antibody expression and targeting within the cell in 1990 [see specific publication cited in (2)], this approach was successfully applied to inhibit the function of several intracellular gene products, including p21Ras (2). The p21Ras molecule appears to be particularly sensitive to intracellular antibody-mediated perturbation of its precise localization to the inner face of the plasma membrane, which is crucial for its activity. Thus, anti-Ras intracellular antibodies with cytoplasmic targeting signals have been shown to divert p21Ras from its intracellular location and to address the antigen–antibody complex to the degradative compartment of the cell (3,4). Such intracellular antibodies are effective irrespective of the epitope recognized, the binding affinity, or the mutant status of the Ras protein broadening the spectrum of intracellular antibodies with potential therapeutic usefulness. In an animal model, intratumor injection of anti-Ras intracellular antibodies expressed by an adenoviral vector resulted in a statistically significant tumor regression, despite a low efficiency of in vivo transduction (5). The striking effects on tumors in that study implicate “by-stander” mechanisms that amplify the direct inhibitory activity of the intracellular antibodies.

Anti-erbB-2 [see references cited in (6)] and p53 (7) intracellular antibodies targeted, respectively, to endoplasmic

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Fig. 1. Intracellular antibody expression in mammalian cells. A) The overall intracellular antibody structure, where a heavy chain variable region (VH) of an antibody connected by a linker to its complementary light chain variable region (VL) (single chain Fv = scFv) is tailed or preceded at the C terminus or N terminus, respectively, by a short targeting signal. B) The presence/absence and the amino acid sequences of some common C-terminal and N-terminal targeting signals (2). These signals allow localization of the intracellular antibody to the nucleus (n), endoplasmic reticulum (er), mitochondria (m), or cytoplasm (c), or to be secreted (s), without affecting the specific binding of the scFv to its target molecule. * = The targeting signal for nuclear localization could be either at the C terminus or at the N terminus. C) Scheme of intracellular antibody compartmentalization mediated by the defined targeting signals.


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RESPONSE

I have read with interest the excellent letter from Canevari, Biocca, and Figini.

The diversion of Ras localization from membrane by intracellular antibodies is indeed a promising and attractive approach to the inhibition of oncogenic ras signaling. The failure to include this approach in my review article was not due to space limitations or a major focus on traditional pharmacologic approaches. It was, rather, due to the decision to focus predominantly on approaches for which phase I human clinical studies had at least commenced.

This explains why other potential approaches with provocative preclinical data such as 1) the ability of trans-farnesylthiosalicylic acid to reduce the amounts of membrane-associated Ras by dislodging Ras protein from its anchorage domains and thus facilitating Ras degradation and reducing the amount of total cellular Ras (1, 2), and 2) Ras C-terminal sequence-specific endoprotease inhibitors such as UM96001, TPCK, and BFCCMK that have been shown to inhibit human cancer cells and selectively induce apoptosis (3) were not included in this review. However, I would like to thank the authors for highlighting the intracellular antibody approach that has yielded interesting results with HER-2/neu targeting and clearly holds promise for the interruption of Ras(p21) signaling.

ALEX A. ADJEI

REFERENCES


NOTE

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