Minimized hormones grow in stature

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Extracellular polypeptide growth factors and cytokines regulate key aspects of cellular proliferation, differentiation, function, and survival. On the basis of their activities, certain of these factors have been tested in the clinic. For example, erythropoietin (EPO) has been used to stimulate production of erythroid cells,[1] and several neurotrophic factors have been exploited to ameliorate neuronal dysfunction and death in disorders of the peripheral nervous system (PNS)[2]. Although not all of these molecules have been successful in trials, polypeptide hormones are generally considered of great clinical potential.

A major limitation to the use of polypeptide therapeutics is their delivery: They must be given intravenously or subcutaneously, are often subject to proteolysis and efficient clearance mechanisms, and only relatively low concentrations may be achieved in tissues of interest. Delivery is especially problematic for the central nervous system (CNS), where the blood-brain barrier severely limits polypeptide entry. One way to overcome these problems is to design small-molecule mimetics that specifically bind and activate signaling receptors. Such molecules would be orally available and achieve high tissue concentrations. In essence, they represent minimized "hormones" with full biological potency. Three recent reports—one by Saragovi and colleagues[3] in this issue, and two by Wrighton and colleagues[4,5] in *Science*—highlight the potential of this approach.

On pages 1120–1122, Saragovi's group report a method to image the nerve growth factor (NGF) receptor TrkA using a minimized cyclic NGF mimetic, C(92-96). TrkA, a tyrosine kinase, and another cell surface receptor, p75, mediate the NGF signaling pathway[6]. TrkA may be a useful marker for cancers such as neuroblastoma, small cell lung carcinoma, and melanoma. It may also be an important therapeutic target in such neurodegenerative diseases as diabetic peripheral neuropathy or Alzheimer's disease.

In their study, Saragovi and colleagues showed that injection of technetium-labeled C(92-96) into tumor-bearing nude mice led to the labeling of E25 tumors that overexpress TrkA, but not of control Y1 or 15N tumors that do not express TrkA. Compared with technetium-labeled monoclonal antibody (5C3), which binds to the extracellular domain of TrkA, the C(92-96) mimetic provided enhanced tumor definition. Given the 100-fold lower affinity of the peptide for TrkA, these results are impressive. Improved resolution may have resulted from the smaller size of the mimetic, allowing more favorable tumor penetration and more rapid clearance from the blood.

C(92-96) (amino acid sequence CTDEKQC) was designed previously by Saragovi's group[7]. It corresponds to a TrkA binding site on NGF in one of the three hydrophilic β loops at the surface of the molecule. The binding site—loop region 92—97—is one of several that have been located on NGF through structure-activity studies of recombinant NGF in which variable regions have been modified or replaced by the analagous region from another neurotrophin. Other binding sites that have been identified include loop regions 29–35 and 43–48, β-strand residues 79–88, and amino-terminal residues 1–9 (ref. 8).

Studies testing the antagonistic/agonistic interaction between small synthetic β-turn mimetics and NGF have gone some way to unraveling function of the contact regions. Early work in our laboratory demonstrated that a peptide corresponding to NGF residues 32–35 could inhibit NGF neurotrophic activity in a sequence-specific and growth factor specific manner[9]. In later studies, linear peptides corresponding to residues 59–67 and to residues 91–100 were also shown to partially inhibit the activity of NGF on sensory neurons[10]. Subsequent research by Ross et al.[11] and Saragovi and colleagues[7] has confirmed that peptide mimetics of loop region 29–35 peptides inhibit NGF activity. In the latter study, it was shown that the mode of action of NGF is also suppressed by NGF loop region 92–97 and that cyclization of the small peptide to more closely mimic the β-turn confers greater inhibitory potency. Significantly, cyclized peptides corresponding to 30–35 and 92–97 inhibited NGF binding to TrkA. However, as yet, no NGF peptides have been found that have TrkA agonist activity. Recent evidence for a direct role of p75 in cell signaling has stimulated interest in discovery of NGF peptide agonists of this receptor[12].

In two separate reports by Wrighton and colleagues[4,5], a similar peptide-mimetic approach to discover ligands for the EPO receptor has been presented. In this case, however, the peptides identified were produced in phage-based random peptide libraries. Using clever selection methods, these authors discovered a disulfide-constrained peptide of 20 amino acids that bears no sequence homology to the native ligand. The K_d for binding to the EPO receptor was 200 nM, compared with 200 pM for EPO itself. Significantly, however, the peptide was shown to specifically activate the EPO receptor and had considerable in vivo EPO activity[4]. Examination of the crystal structure of the peptide bound to the extracellular domain of the EPO receptor revealed that the peptide was present as a dimer and that it formed a complex with two receptor molecules. Remarkable features of the structure were that a portion of each peptide monomer interacted with both receptor molecules and that the sites for peptide binding to the receptor were likely to be a subset of those used by the natural ligand[5].

Taken together, these experiments demonstrate that a relatively small number of ligand-receptor interactions may be required to produce specific, high-affinity receptor binding. Importantly, when properly arrayed, as in the EPO peptide mimetic, they can be used to create a functional epitope. The growing list of studies demonstrating the efficacy of small molecules that resemble specific domains of multideterminant ligands goes some way toward silencing skeptics who suggest that the extensive binding surfaces that characterize ligand-receptor interactions and the requirement for receptor dimerization and oligomerization simply cannot be addressed using this technology.

Small nonpeptide agonists of growth factor and cytokine receptors are not yet a reality. But peptide-based strategies to discover "minimized hormones," such as those of Saragovi's group and Wrighton and colleagues, may well be an important step in the discovery of such reagents.

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