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MINI REVIEW

# Translational research of a novel humanized epidermal growth factor receptor-related protein: a putative inhibitor of pan-ErbB

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**Abstract**
*Purpose* The ErbB family members are protein tyrosine kinases, which play a crucial role in the signal transduction pathways that regulate key cellular functions. Overexpression of the ErbB family members is associated with oncogenicity, metastatic potential, cell proliferation, apoptosis, angiogenesis, and prognosis in cancer. Molecular-targeted therapies centered on the ErbB signaling pathway are the currently promising anti-cancer therapies.
*Methods* We reviewed the literature to summarize the current knowledge of epidermal growth factor receptor (EGFR)-related protein (ERRP) and determine the potential of this protein to be translated into a molecular-targeting treatment for cancer.
*Results* *ERRP* isolated from rat gastroduodenal mucosa is a pan-ErbB inhibitor that targets multiple members of the ErbB family both in vitro and in vivo. Sequestration of ErbB ligands by ERRP results in the formation of inactive ErbB heterodimers and subsequent attenuation of signaling pathways activated by ErbB. We suggest a strategy to develop a humanized ERRP protein based on activity of rat EERP in vitro.
*Conclusions* As rat ERRP protein is expected to generate an immune response in humans, we propose a hypothesis that a humanized version of ERRP has potential therapeutic value for cancer patients.

**Keywords** Epidermal growth factor receptor · Epidermal growth factor receptor-related protein · Pan-ErbB inhibitor · Anti-neoplastic

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## Background

The ErbB family of receptor tyrosine kinases contains four members, epidermal growth factor receptor (EGFR; ErbB1), HER-2 (ErbB2), HER-3 (ErbB3), and HER-4 (ErbB4), which share a similar primary structure and are expressed widely in human tissues. Binding of receptor-specific ligands to the EGFR, HER-3 and HER-4 ectodomains results in the formation of homodimeric and heterodimeric complexes with kinase activity, to which HER-2 is recruited to as a preferred partner. HER-3 has impaired kinase activity due to substitutions in crucial residues in the tyrosine kinase domain and contains six docking sites for the p85 adaptor subunit of phosphatidylinositol 3-kinase (PI3 K). When dimerized with another ErbB receptor, HER-3 becomes phosphorylated and functions as a signaling entity [1].

Head and neck, colorectal, lung, pancreas, bladder, and breast cancers overexpress EGFR during development and progression [2, 3]. Overexpression or activation of EGFR is associated with resistance to cytotoxic drugs and shorter survival. HER-2 overexpression is observed in approximately 20% of breast cancer samples and is related to metastasis and poorer prognosis [4]. HER-2 expression is also associated with a poorer prognosis in gastric,

esophageal, ovary, colon, and bladder cancers [5–8]. Although the role of HER-3 and HER-4 in the development and progression of cancer is less well defined, increased expression of these receptors has been observed in breast and gastrointestinal cancer [9].

In light of the evidence that the ErbB receptor family plays an important role in cancer, interference with growth factor receptor activation and/or intracellular growth factor-activated signal transduction pathways represents a promising strategy for molecular-targeted anticancer therapies. A number of inhibitors targeting the ErbB receptors have been developed, in particular, the monoclonal antibodies, cetuximab and trastuzumab, and small molecule tyrosine kinase inhibitors, gefitinib and erlotinib, which have shown promise as chemotherapeutic agents. Although cetuximab and trastuzumab can be initially successful, failure occurs in some patients due to co-expression of multiple ErbB family members. Targeting a single ErbB receptor can often lead to activation of signaling by other ErbB receptors, resulting in the development of resistance. Therefore, it is imperative that strategies are developed to target multiple members of the ErbB family, as such agents are anticipated to display a superior efficacy. Lapatinib, an oral receptor tyrosine kinase inhibitor that targets both EGFR and HER-2 [10], is effective in trastuzumab-naive and trastuzumab-refractory HER-2-positive advanced breast cancer patients, with response rates of 24 and 8%, respectively [11].

## Cloning and characteristics of *ERRP* cDNA

In an attempt to develop a pan-ErbB inhibitor, *EGFR-related protein* (*ERRP*) was isolated from rat gastroduodenal mucosa [12]. Analysis of the 1,958 bp full-length *ERRP* cDNA clone (Genbank accession no. AF187818) revealed a 227 bp 5′-untranslated region (UTR) containing an open reading frame encoding 479 amino acids and a 290 bp 3′-UTR. *ERRP* cDNA shares 85–90% nucleotide homology with the external domain of rat *EGFR* and 50–60% homology with *ErbB-2, ErbB-3,* and *ErbB-4* [12]. Though the human homolog of rat *ERRP* has not yet been isolated, rat *ERRP* shows approximately 85% homology to the extracellular domain of human *EGFR* [13]. *ERRP* contains a 5′ sequence encoding a portion of the ligand-binding domain (subdomains I–III) of rat *EGFR* and a 3′ sequence identical to rat *peptidase D* (*PEPD*); the *EGFR* and *PEPD* genes are localized to rat chromosomes 14 and 1, respectively. ERRP contains 479 amino acids and is a 53–55 kDa secretory protein, possessing three of the four extracellular ligand-binding domains of EGFR, which are responsible for the subsequent homo/heterodimerization with other ErbB members. ERRP contains a region of 27 amino acids (nucleotides 1580–1661) located at the carboxy terminus, referred to as the "U" region, which shows no homology to any known protein sequence [14].

## ERRP acts as a pan-ErbB inhibitor both in vitro and in vivo

ERRP is a pan-ErbB inhibitor, which targets multiple members of the ErbB family. We hypothesized that an ERRP analog could be a potential therapeutic agent for cancer. Transfection of *ERRP* cDNA into the colon cancer cell lines HCT116 and Caco-2 inhibited proliferation in matrix-dependent and matrix-independent systems. *ERRP*-induced inhibition was associated with attenuation of EGFR tyrosine phosphorylation and tyrosine kinase activity [12]. To further determine the therapeutic potential of ERRP, affinity-purified recombinant soluble ERRP fusion protein was generated using a Drosophila expression system [14]. Recombinant ERRP inhibited proliferation in a dose-dependent manner and induced apoptosis in prostate, colon, gastric, pancreatic, breast, and non-small cell lung cancer cells in vitro. These effects were linked to inhibition of EGFR signaling and attenuation of downstream Akt, mitogen-activated protein kinase (MAPK), and nuclear factor (NF-$\kappa$B) signaling [1, 9, 14–16]. ERRP attenuates basal and ligand-induced (TGF-$\alpha$, HB-EGF, and heregulin) EGFR and Her-2 activation in a variety of epithelial cancers, suggesting ERRP has a pan-ErbB inhibitory property. Studies of xenograft models in SCID mice demonstrate that recombinant ERRP can inhibit the growth of colon and pancreatic cancers in vivo [14, 17]. In these experiments, ERRP was effective at 25 μg/kg and was tolerated up to 100 μg/kg, without signs of toxicity in SCID mice. Withdrawal of ERRP administration led to tumor regrowth, but this was at significantly reduced rates [14, 17]. Thus, evidence suggests that ERRP could act as a potential therapeutic agent in a wide variety of epithelial cancers.

## The mechanism by which ERRP inhibits ErbB signaling

The ability of soluble ErbB proteins to inhibit ligand-induced receptor activation and downstream signaling pathways is well characterized [18, 19]. In addition, expression of endogenous soluble ErbB isoforms has been demonstrated in human tissues [20, 21]. It is reported that a truncated EGFR isoform lacking the receptor extracellular domain IV exists, which can bind EGF and TGF-$\alpha$ with higher affinity than full-length EGFR [22]. ERRP, which lacks most of the extracellular domain IV, also binds TGF-$\alpha$ and is expected to effectively and preferentially sequester other ErbB ligands [13]. Sequestration of EGFR ligands by

ERRP results in the formation of inactive ERRP and EGFR heterodimers, leading to decreased availability of ligands to bind and activate EGFR, and subsequently attenuating EGFR-activated signaling pathways. This mechanism may partly explain the attenuation of EGFR function in the presence of ERRP.

## Hypothesis

We propose that humanized ERRP has potential therapeutic value for many cancer patients.

## Testing the hypothesis

Rat EPPR protein would be expected to generate an immune response in humans. Marginal immune activation was observed after analysis of human peripheral blood lymphocytes CD69 expression in response to recombinant rat ERPP in vitro [23]; however, we have reservations about the potential therapeutic value of rat ERRP in humans. Due to the apparent chimeric nature of ERRP and the lack of evidence to indicate the existence of a human *ERRP* homolog, the *ERRP* coding sequence (CDS) at 1–1,340 bp could be substituted with the human *EGFR* CDS from 247 to 1,586 bp (Genbank accession no. NM-005228.3). Additionally, the region encoding the 446 amino acids at the 5′ end of *ERRP* could be humanized and the *ERRP* 3′ end U region could be included or left out. A humanized recombinant ERRP, referred to as ErbB inhibitory protein (EBIP), has recently been generated and can inhibit the growth of breast cancer cells in SCID mice xenograft models [24]; however, it is unknown whether the humanized ERRP 5′ 446 amino acids are effective without the 3′ U region. As previously discussed, the anti-neoplastic activity of ERRP is mediated by competitive binding of TGF-α and other ErbB ligands [13]. It is reported that a 501 amino acid of aminoterminus-truncated ectodomain of human EGFR can bind EGFR ligands, such as EGF and TGF-α, with 13- to 14-fold higher affinity than the full-length EGFR ectodomain [25]. We deduce that ligand-binding activity is completely mediated by the 5′ end of rat ERRP encoding the ligand-binding domain and speculate that the 3′ U ERRP region is not required for ligand binding. Removal of the EPPR 3′ U region may increase the ligand-binding affinity or enhance stability, as the U region shows no nucleotide homology to any human gene [13] or known protein sequence [14]. If the 3′ U region is essential for ERRP ligand-binding activity, it may be preferable to synthesize a humanized peptide with similar characteristics, instead of using the rat 3′ U region, which may stimulate immune rejection in humans.

## Conclusion

In conclusion, we believe that humanized ERRP, a novel pan-ErbB inhibitor, has potential utility as a therapeutic agent for a wide variety of cancers. Further investigation and rigorous validation are required to support the potential therapeutic value of humanized ERRP.

**Conflict of interest** None.

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