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Recombinant retroviral vectors that express EGF, TGF alpha, NRG2 beta, and the NRG2 beta Q43L mutant V.1

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Abstract

Here we describe the construction of recombinant retroviral expression vectors based on pLXSN-HygR that drive the ectopic expression of the Epidermal Growth Factor (EGF), Transforming Growth Factor alpha (TGFalpha), Neuregulin 2beta (NRG2beta), or the NRG2beta Q43L mutant protein.

Introduction

1 Elevated signaling by members of the epidermal growth factor receptor (EGFR/ErbB) family of receptor tyrosine kinases contributes to numerous human malignancies. This elevated signaling may be due to gain-of-function mutations in the receptor genes, increased receptor gene transcription, or elevated ligand expression [1-11]. Because these receptors are tractable targets for therapeutic intervention [12-32], there is much interest in tools that can be used to study these mechanisms of elevated signaling and the biological consequences of elevated signaling.

2 Here we describe the construction of recombinant retroviral expression vectors based on pLXSN-HygR (pLXSN-HygR.dna 56KB) [33] that drive the ectopic expression of the Epidermal Growth Factor (EGF) [34], Transforming Growth Factor alpha (TGFalpha) [34], Neuregulin 2beta (NRG2beta) [35-40], or the NRG2beta Q43L mutant protein [39, 40].

Methods

3 **Construction of pLXSN-HygR-EGF**

3.1 We have previously described the construction of pENTR-EGF-Short (pENTR-EGF-Short.dna 32KB) [34]. This plasmid encodes the soluble, mature form of EGF. This EGF coding sequence is flanked in frame on the 5' end by a sequence that encodes a BiP signal sequence to facilitate protein trafficking. The EGF coding sequence is flanked in frame on the 3' end by V5 and His6 tags to facilitate immunodetection and purification. The sequence of the entire BiP-EGF-V5-His6 fusion protein is shown in **Figure 1** (below).

Figure 1.

BiP-EGF-V5-His6
MKLCILLAVVAFVGLSLGRSNSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRDLKWWELRPRFEGKPIPNPLLGLDSTRTGHHHHHH

BiP-TGFalpha-Short-V5-His6
MKLCILLAVVAFVGLSLGRSVVSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGARCEHADLLAPRFEGKPIPNPLLGLDSTRTGHHHHHH

BiP-NRG2beta-V5-His6
MKLCILLAVVAFVGLSLGRSSGHARKCNETAKSYCVNGGVCYYIEGINQLSCKCPVGYTGDRCQQFAMVNFSKHLGFELKEAEELYQKPRFEGKPIPNPLLGLDSTRTGHHHHHH

BiP-NRG2beta-Q43L-V5-His6
MKLCILLAVVAFVGLSLGRSSGHARKCNETAKSYCVNGGVCYYIEGINQLSCKCPVGYTGDRCLQFAMVNFSKHLGFELKEAEELYQKPRFEGKPIPNPLLGLDSTRTGHHHHHH

The BiP sequence is underlined, the EGF sequence is doubly underlined, and the V5 sequence is underlined.

3.2 We amplified the BiP-EGF-V5-His6 coding sequence from pENTR-EGF-Short using primers Modified-BiP-Primer and Modified-His6-Primer, as shown in **Figure 2** (below – also see pENTR-EGF-Short.dna 32KB).

Figure 2.

Modified-BiP-Primer
5′gttaacctcgagatgaagttatgcatattactggccgtc

Modified-His6-Primer
5′gttaacggatccctcaatggtgatggtgatgatgaccg

The resulting amplicon is predicted to be approximately 328 bp in length and contain a unique XhoI site immediately upstream of the BiP coding sequence and a unique BamHI site immediately downstream of the His6 coding sequence. Thus, the amplicon was digested with XhoI and BamHI and was ligated to the 6412 bp fragment of pLXSN-HygR-ERBB2 pLXSN-HygR-ERBB2.dna 72KB), which encodes the pLXSN-HygR vector sequences. We used standard molecular biology techniques to complete this subcloning. Candidate clones were validated by restriction mapping and next-generation DNA sequencing (NGS), resulting in pLXSN-HygR-EGF (pLXSN-HygR-EGF.dna 53KB).

4 **Construction of pLXSN-HygR-TGFalpha-Short**

4.1 We have previously described the construction of pENTR-TGFalpha-Short (pENTR-TGFalpha-Short.dna 30KB) [34]. This plasmid encodes the soluble, mature form of TGFalpha. This TGFalpha coding sequence is flanked in frame on the 5′ end by a sequence that encodes a BiP signal sequence to facilitate protein trafficking. The TGFalpha coding sequence is flanked in frame on the 3′ end by V5 and His6 tags to facilitate immunodetection and purification. The sequence of the entire BiP-TGFalpha-Short-V5-His6 fusion protein is shown in **Figure 1** (above). The BiP sequence is underlined, the TGFalpha-Short sequence is doubly underlined, and the V5 sequence is underlined.

4.2 We amplified the BiP-TGFalpha-Short-V5-His6 coding sequence from pENTR-TGFalpha-Short using primers Modified-BiP-Primer and Modified-His6-Primer (**Figure 2** above– also see pENTR-TGFalpha-Short.dna 30KB). The resulting amplicon is predicted to be approximately 319 bp in length and contain a unique XhoI site immediately upstream of the BiP coding sequence and a unique BamHI site immediately downstream of the His6 coding sequence. Thus, the amplicon was digested with XhoI and BamHI and was ligated to the 6412 bp fragment of pLXSN-HygR-ERBB2 (pLXSN-HygR-ERBB2.dna 72KB) which encodes the pLXSN-HygR vector sequences. We used standard molecular biology techniques to complete this subcloning. Candidate clones were validated by restriction mapping and next-generation DNA sequencing (NGS), resulting in pLXSN-HygR-TGFalpha-Short (pLXSN-HygR-TGFalpha-Short.dna 53KB).

5 Construction of pLXSN-HygR-NRG2beta and pLXSN-HygR-NRG2beta-Q43L

5.1 We have previously described the construction of pMT-BiP-NRG2beta-V5-His6 (pMT-BiP-NRG2beta-V5-His6.dna 46KB) [36, 37]. This plasmid encodes the soluble, mature form of NRG2beta. This NRG2beta coding sequence is flanked in frame on the 5’ end by a sequence that encodes a BiP signal sequence to facilitate protein trafficking. The NRG2beta coding sequence is flanked in frame on the 3’ end by V5 and His6 tags to facilitate immunodetection and purification. The sequence of the entire BiP-NRG2beta-V5-His6 fusion protein is shown in **Figure 1** (above). The BiP sequence is underlined, the NRG2beta sequence is doubly underlined, and the V5 sequence is underlined.

5.2 We amplified the BiP-NRG2beta-V5-His6 coding sequence from pMT-BiP-NRG2beta-V5-His6 using primers Modified-BiP-Primer and Modified-His6-Primer (**Figure 2** above – also see pMT-BiP-NRG2beta-V5-His6.dna 46KB). The resulting amplicon is predicted to be approximately 373 bp in length and contain a unique XhoI site immediately upstream of the BiP coding sequence and a unique BamHI site immediately downstream of the His6 coding sequence. Thus, the amplicon was digested with XhoI and BamHI and was ligated to the 6412 bp fragment of pLXSN-HygR-ERBB2 (pLXSN-HygR-ERBB2.dna 72KB), which encodes the pLXSN-HygR vector sequences. We used standard molecular biology techniques to complete this subcloning. Candidate clones were validated by restriction mapping and next-generation DNA sequencing (NGS), resulting in pLXSN-HygR-NRG2beta (pLXSN-HygR-NRG2beta.dna 54KB).

5.3 We constructed pLXSN-HygR-NRG2beta-Q43L, which encodes the Q43L mutant of NRG2beta, in an identical manner, except we used pMT-BiP-NRG2beta-Q43L-V5-His6 (pMT-BiP-NRG2beta-Q43L-V5-His6.dna 46KB) as the template for the PCR amplification reaction. The sequence of the BiP-NRG2beta-Q43L-V5-His6 fusion protein is provided in **Figure 2** (above). The Q43L mutant is indicated by red text and yellow highlighting. The sequence of pLXSN-HygR-NRG2beta is provided in pLXSN-HygR-NRG2beta-Q43L.dna 53KB .

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