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Recombinant retroviral expression vectors that encode dominant-negative alleles of EGFR and ERBB2

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Abstract

EGFR and *ERBB2* mutant alleles that encode proteins that lack tyrosine kinase activity typically possess a dominant-negative genotype. Thus, here we describe the construction of recombinant retroviral expression vectors based on pLXSN and pLXSN-HygR. These recombinant retroviral vectors express *EGFR* K721A and *ERBB2* K753A mutant alleles that encode proteins deficient for kinase activity.

Troubleshooting

Introduction

1 We have described recombinant retroviral vectors based on pLXSN that encode a neomycin resistance gene and wild-type *EGFR* and *ERBB2* alleles. Therefore, mammalian cells infected with these constructs are resistant to the antibiotic G418 [1].

2 We have also described recombinant retroviral vectors based on pLXSN-HygR that encode a hygromycin resistance gene and wild-type *EGFR* and *ERBB2* alleles. Therefore, mammalian cells infected with these constructs are resistant to hygromycin [2].

3 *EGFR* and *ERBB2* mutant alleles that encode proteins that lack tyrosine kinase activity typically possess a dominant-negative genotype. Thus, here we describe the construction of recombinant retroviral expression vectors based on pLXSN and pLXSN-HygR. These recombinant retroviral vectors express *EGFR* K721A [3-6]and *ERBB2* K753A [7, 8] mutant alleles that encode proteins deficient for kinase activity.

Methods

4 **Construction of pLXSN derivatives that encode the *EGFR* K721A or *ERBB2* K753A mutant alleles**

4.1 We have previously described the construction of pLXSN-EGFR [1, 9]. We performed traditional site-directed mutagenesis of pLXSN-EGFR to introduce the AA4114GC (A4114G and A4115C) mutations, thereby changing the Lys721 codon (AAA) to an alanine codon (GCA). Simultaneously, we introduced the TCCC4101ACCG (T4101A and C4104G) mutations, thereby creating an AgeI site but silently affecting the Ile716 and Pro717 codons (pLXSN-EGFR-K721A.dna 73KB). Unfortunately, the primer sequences used to create these mutations have been lost. The predicted sequence of the pLXSN-EGFR-K721A clone has been confirmed by next-generation DNA sequencing (NGS).

4.2 We have previously described the construction of pLXSN-ERBB2 [1, 9]. We performed traditional site-directed mutagenesis of pLXSN-ERBB2 to introduce the AA4015GC (A4015G and A4016C) mutations, thereby changing the Lys753 codon (AAA) to an alanine codon (GCA). Simultaneously, we introduced the A4035G mutation, creating a TaiI (HpyCH4IV) site but silently affecting the Thr759 codon (pLXSN-ERBB2-K753A.dna 82KB). Unfortunately, the primer sequences used to create these mutations have been lost. NGS has confirmed the predicted sequence of the pLXSN-ERBB2-K753A clone.

5 **Construction of pLXSN-HygR derivatives that encode the *EGFR* K721A or *ERBB2* K753A mutant alleles**

5.1 We have previously described the construction of pLXSN-HygR-ERBB2 (pLXSN-HygR-ERBB2.dna 72KB) [2]. We used conventional subcloning techniques to replace the 3233 bp AvrII-AseI fragment of pLXSN-EGFR-K721A, which contains the neomycin resistance gene, with the corresponding 3607 bp AvrII-AseI fragment of pLXSN-HygR-ERBB2, which contains the hygromycin resistance gene. This approach resulted in pLXSN-HygR-EGFR-K721A (pLXSN-HygR-EGFR-K721A.dna 82KB), whose predicted sequence has been confirmed by NGS.

5.2 We used conventional subcloning techniques to replace the 3654 bp NotI-AseI fragment of pLXSN-ERBB2-K753A, which contains the neomycin resistance gene, with the corresponding 4028 bp NotI-AseI fragment of pLXSN-HygR-ERBB2, which contains the hygromycin resistance gene. This approach resulted in pLXSN-HygR-ERBB2-K753A (pLXSN-HygR-ERBB2-K753A.dna 70KB), whose predicted sequence has been confirmed by NGS.

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