A Novel Renilla Luciferase/Epidermal Growth Factor Fusion Protein as an Optical Molecular Probe for Cancer Imaging

Andreas M. Loening Ramasamy Paulmurugan Anna M. Wu Sanjiv S. Gambhir
Crump Institute for Molecular Imaging, UCLA School of Medicine Department of Radiology and the Bio-X Program, Stanford University

INTRODUCTION

Our lab is working on developing novel probes for imaging receptor expression using in vivo optical techniques. One potential target, epidermal growth factor (EGF) receptor, is up-regulated in many forms of cancer [1]. In light of this, we have pursued the construction and validation of a Renilla Luciferase (RLuc)/EGF fusion protein as a probe for the study of the EGF receptor system in vivo.

CONSTRUCTS

Wild Type Constructs

IN VITRO EVALUATION

Specificity

Specificity of the fusion protein was assessed by comparing binding of RLuc-EGF to RLuc on both A431 human epidermoid carcinoma cells (high EGF receptor expression) and NIH 3T3 murine fibroblasts (negative control). Cells were plated at 1 x 10^5/well in 24 well plates and allowed 24 hours to attach. 8000 RLU’s/well of either fusion or control protein was then applied and allowed to attach. The experiment was repeated with the RLucC124A-EGF protein, but this enhanced activity could not be demonstrated to be specific to binding of the EGF receptor.

C124A Constructs

The above constructs were assembled via PCR cloning. CMV-hRLuc plasmid (Promega) was used as the initial template, and the DNA sequence encoding the 53 amino acids of mature human EGF was extended onto either the 5’ or 3’ end of the hRLuc gene, with single glycines (dark blue) used as spacers. The pelB leader sequence was then appended to the 5’ end to provide a signal for protein export to the bacterial periplasm.

The final fusion genes were then cloned into the pBAD/Myc-HisA plasmid (Invitrogen) such that the Myc epitope and 6xHistidine tag were attached to the fusion construct. The pBAD plasmid is a bacterial expression vector in which the protein of interest is under the control of an inducible arabinose promoter.

Purification

Protein production was performed in E. coli LMG 194 cells grown at 32°C. Cultures were allowed to come to an OD_600 of 0.7, and arabinose was added to a final concentration of 0.2%. Two hours later, cells were harvested and the periplasm extracted by osmotic shock using the method of Neu and Heppel [3]. The periplasm was brought to 1 mM PMSF, and then purified by nickel affinity chromatography (Ni-NTA Superflow, Qiagen). The chromatography buffer consisted of 300 mM NaCl and 20 mM HEPES at pH 8, with 20 mM imidazole in the loading and washing steps and 250 mM imidazole in the elution step. Human serum albumin was added to 1% as a carrier protein, and the elution was then desalted using a PD-10 column. This partially purified protein solution was stored for later use in 20% glycerol at ~80°C.

IN VIVO EVALUATION

Distribution of RLucC124A

The distribution of RLucC124A was assessed in non-tumor bearing athymic (nude) mice to check the persistence of Renilla activity in vivo. RLucC124A protein (~250 x 10^6 RLU’s) was injected iv at t = 0, and iv injections of 10 µg coelenterazine immediately preceded each imaging time point.

A representative mouse is shown below. The results demonstrated that imaging of iv injected Cys124Ala mutated Renilla is feasible over a matter of hours.

CONCLUSION

A bifunctional RLuc-EGF fusion protein has been evaluated in vitro, with specificity demonstrated by cell binding assays and competitive binding experiments with native EGF.

In vivo and in vitro stability of Renilla luciferase was shown to markedly improve following the incorporation of the Cys124Ala mutation.

Following iv administration, mutant Renilla luciferase activity could be imaged in vivo for up to 2.5 hrs.

Tumor bearing mice showed enhanced luciferase activity in the tumor following iv injection of the RLucC124A-EGF protein, but this enhanced activity could not be demonstrated to be specific to binding of the EGF receptor.

References


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