International Journal of Peptide Research and Therapeutics (2020) 26:1399–1407
https://doi.org/10.1007/s10989-019-09941-0

Optimization of Anti-CXCL10 Nanobody Expression Using Response Surface Methodology and Evaluation of its Anti-metastatic Effect on Breast Cancer cells

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Received: 16 April 2019 / Revised: 30 July 2019 / Accepted: 28 September 2019 / Published online: 11 October 2019

Abstract
Some chemokines and chemokine receptors play important roles in various types of autoimmune diseases, infectious diseases and cancer metastasis. Hence, production of neutralizing antibodies against them are under active investigation. We previously developed a nanobody against CXCL10, designated as 3NB12, which can be expressed by *E. coli* cells. In the present study, we carried out a detailed study to optimize its expression using response surface methodology based on manipulation of three variables, including temperature, IPTG concentration, and post-induction time. In addition, upon expression and purification of the nanobody, it was also used to evaluate its inhibitory effects on migration of CXCR3 overexpressing MDA-MB-231 breast cancer cells. Seventeen experiments were designed. Total protein of the designed experiments was assayed by SDS-PAGE, followed by size exclusion chromatography to qualify and quantify the relative concentration of the nanobody in the optimized expression condition. The model designed according to the Box Behnken method predicted maximum 3NB12 expression at 28.5 °C, a post-induction time length of 15 h, and 0.9 mM IPTG. Chemotaxic assessment results showed that 3NB12 potently inhibits migration of the cells which has an important role in methastasis of breast cancer. Taken together, a reasonable amount of the nanobody could be produced according to the present study for being used in later in vitro and in vivo studies to further evaluate its anti metastatic and also anti-inflamatory effects.

Keywords Nanobody · Chemokine · CXCL10 · CXCR3 · Optimization of expression · Response surface methodology

Introduction

Despite their rapid trust-gaining, the generation, production, functionalization, and application of full-length antibodies have limited due to some bottelnecks such as their immunogenicity,, low penetration into dense tissues, large sizes, high production cost, and production difficulties because of structural specificities such as post-translational glycosylation, and inter- and intramolecular disulfide bonds. Therefore, production of novel classes of recombinant antigen-binding proteins lacking these limitations might alleviate some of these concerns. The variable fragments of Camelid heavy-chain only antibodies (HcAbs), called nanobodies, are notable examples of these proteins. Nanobodies are characterized by unique physical and biochemical properties including small size (~15 kDa, 4 nm long, and 2.5 nm wide), high affinity, specificity, solubility, and stability, thermal and chemical resistance as well as ease of cloning and production

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