ABSTRACT

PRODUCTION OF PHARMACEUTICALLY IMPORTANT PROTEIN IN NON-CONVENTIONAL YEASTS

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TNFα is an pleotropic, important pro-inflammatory cytokines of immune system, produced by immune cells. It is imperative for the induction of inflammation by immune cells, plays a significant role in development of lymphoid tissues in pathogenesis of immune disorders and tumor development. Increased levels of TNFα have been reported at the inflammation sites of rheumatoid arthritis. Neutralization of TNFα by monoclonal antibody results in the reduction of inflammatory symptoms. Currently there are five anti-TNFα drugs approved by FDA for different conditions like rheumatoid arthritis, psoriasis and Crohn's disease. These drugs are effective for the treatment of patients with autoimmune diseases. However, the usage of these large molecules as inhibitors has been limited by high production cost and potential side effects.

Single chain antibody fragments (scFv) is a small antibody fragment which consist of variable region of heavy and light chain of the antibody joined together by a peptide linker. scFv retains the affinity and specificity of the parent antibody and

being smaller in size it is easy to manipulate in terms of affinity and specificity. Phage display system has emerged as a new and promising platform for the isolation and identification of monoclonal antibodies with desirable properties. Considering the merits of scFv and the ease to isolate them using phage display technology, anti TNF α scFv fragment was isolated using these techniques.

Tomlinson scFv phage display libraries were used for the isolation of scFv against hTNF α. Clones obtained in three rounds of selection were further characterized for their affinity and specify for hTNFα. Cell based assay was carried out to assess the functional activity of the protein. Protein Isolated from positive clones effectively neutralized the cytotoxic effect of hTNF α on L929 cell line. Affinity enhancement of the selected clone was carried out by error prone PCR followed by a screening using yeast display technique. Stringent screening from yeast display library resulted in an affinity improvement of 11nM, which is as competitive as previous studies carried out in this area of research.

Anti-TNFα binding clone was expressed in *Pichia pastoris* system. Single and double copy expression cassette were generated and transformed in *P. pastoris* strain GS115 and X33. Transformants were confirmed using molecular biology techniques and further characterized based on the protein expression. A stable expression of 501 mg/L of anti- TNFα scFv was recorded from the selected transformant. *P.pastoris* expressed protein were assayed for their functionality on

HeLa cell line. Results indicated that the expressed protein is efficiently able to neutralize the hTNF α in cell lines.