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BIOPRODUCTION OF HUMAN DEFENSINS ANTIMICROBIAL PEPTIDES IN TRANSGENIC PLANTS

LALIT LATA JHA*[1], DARSHIKA SHAH[1] AND RAJESH KS[1]

Department of pharmaceutics, Parul Institute of Pharmacy, Vadodara, India.

LALIT LATA JHA
Department of pharmaceutics, Parul Institute of Pharmacy, Vadodara, India.

ABSTRACT

Human defensins are small antimicrobial and cytotoxic peptides produced by neutrophils and epithelial cells. They exhibit broad range of antimicrobial properties and are thought to be ideal therapeutic agents because of their potential ability to circumvent the problems of acquired resistance often observed with other antimicrobial therapies. Therefore studies have been started to clone and express these peptides in bacterial and plant systems. There are certain major hurdles of producing the defensins peptides in bacteria like toxic action of the peptide on the host cells, costs of production by these technologies and scale up is also very difficult. Transgenic plants as bioreactors pave a new way of producing recombinant therapeutic defensins peptides. The present review paper describes an overview of human defensins including its classification, molecular structure, biosynthesis, localization, mechanism of action, potential medical application and production of human defensins by r-DNA technology in bacterial and plant systems.

KEYWORDS

Antimicrobial peptides, Human Defensins and Transgenic plants

INTRODUCTION

AntiMicrobial Peptides (AMPs) are the cytolytic peptides that are produced by the living organisms of all types (vertebrates and invertebrates) including humans in response to infection and exert antibiotic like action against pathogenic microorganisms, e.g. bacteria, fungi, viruses and parasites[1-2]. They are produced on epithelial surface and in phagocytic cells and plays an important role in innate and adaptive defence systems[3]. Thus they are having both offensive and defensive function. **Human Defensins** are small, cationically charged, cysteine-rich endogenous antibiotic peptides with a molecular weight of 4-5 kDa[4]. In recent years considerable research has been started on human defensins because of their potential medical and pharmaceutical application[5-8].

CLASSIFICATION:

The eukaryotic antimicrobial peptides are divided into four groups according to their structural features: Cysteine- free α helices, extended Cysteine- free α helices with a predominance of one or two amino acids, loop structures with one intramolecular disulfide bond, and β sheet structures which are stabilized by two or three intermolecular disulfide bonds[9-10]. **Human** defensins are part of the fourth group. The human defensins are classified into three main groups according to their structural differences: the α defensins, β defensins and θ defensins[11]. To date six α defensins have been identified in humans. Four of these designated human neutrophil peptides (HNP) 1, 2 , 3 & 4, form part of the armory of neutrophils, where they participate in systemic innate immunity. The remaining two, Human Defensins (HD) 5 and 6, are expressed in intestinal Paneth cells and probably contribute to innate defense of the GI mucosal surface[12]. The β defensins have been isolated from both leukocytes and epithelial cells and classified into four main types as Human β defensins (HBD) (HBD1-to HBD-4)[13].

MOLECULAR STRUCTURE

Human defensins are 29-35 amino acids cationic peptide, including six invariant cysteins whose intramolecular disulfide bonds cyclise and stabilise them in a complexly folded, triple stranded β sheet configuration. The peptides HNP-1, HNP-2, and HNP-3 are rich in cystine, arginine, and aromatic residues, but are devoid of sulfhydryl groups and carbohydrate moieties[14-16].

BIOSYNTHESIS:

The neutrophil granulocytes is the most numerous leukocytes in peripheral blood. The development from a multipotent progenitor cell to a mature neutrophil takes place in the bone marrow over a period of 10-14 days. Human α defensins (HNPs) are produced from 93-95 amino acids precursors by stepwise, tissue specific, proteolytic removal of 64 amino terminal residues to produce the mature defensins[17-20]. The early proteolytic processing events include two sequential cleavages, each removing 19 amino terminal aa residues, that yield 75 and 56 aa prodefensins respectively. The first cleavage is the removal of a signal peptide and second by preaspartate proteolytic cleavage in the bone marrow[21-24]. The subsequent processing steps occurs in the peripheral blood neutrophils and convert these 56 aa prodefensins to mature (30 aa) HNP-1 and HNP-3.

LOCALISATION:

Human defensins peptides have been found to be in the granules of phagocytes, intestinal paneath cells, on epithelial surfaces of the intestine and trachea[25]. Microbes in the phagocytic vacuoles of granulocytes and certain macrophages encounter high concentrations of defensins[26]. Increased transcription of defensin genes and stimulus dependent release of pre-synthesized defensin-containing cytoplasmic granules contribute to the local antimicrobial response[27]. The α human defensins constitute more than 5% of total cellular protein in human Polymorpho Neutrophils (PMN). They comprise 30-50% of the total protein in the azutrophic granules of human neutrophils, the most abundant is HNP1[28-30]. Human neutrophil contain large amounts of three α defensins (HNP-1 to HNP-3), and a smaller amounts of a fourth, HNP-4. HNP 1-3 are detected in bone marrow cells by Northern blot analysis and in peripheral leucocytes by the reverse transcription-polymerase chain reaction[31-34]. HNPs 1-3 are absent from mononuclear phagocytes systems such as peripheral monocytes, alveolar macrophages and Kuffler cells in liver[35]. They are specific to the cells of neutrophil lineage and functions as microbicidal agents in the neutrophil mediated defense system[36-41]. They are released upon neutrophil stimulation. Endogenous molecular forms of HNPs are isolated from normal bone marrow, plasma and peripheral blood neutrophils. Immunoproxidase stains revealed HNP 1-3 to have a granular localization in the neutrophil's cytoplasm by light microscopy. Frozen thin section immunogold transmission electron microscopy showed HNP 1-3 to be localized in azurophic granules[42-49]. Monocytes and macrophages generally lack defensins, but they release messengers that induce the synthesis of β defensins in epithelial cells[50-59].

MECHANISM OF ACTION:

Human defensins AMPs are membrane active, They act by disrupting negatively charged target cell surface molecule to which they are electrostatically attracted[60]. Hydrophobic face of the amphipathic structure of defensins AMPs disrupts the lipid bilayer of bacterial cell wall; causing membrane depolarisation which leads to cell death[61].

DIFFERENT THERAPEUTIC APPLICATIONS OF DEFENSINS:

All defensins are active against majority of gram positive and gram negative bacteria and thought to be an ideal candidate to be developed as an antimicrobial agent. Studies have suggested that defensins can be used as an adjunct to antitubercular drugs. The studies have shown that the combination of HNP-1, isoniazid, and rifampicin is active against *Mycobacterium tuberculosis H37Rv* in vitro, ex vivo, and in vivo, and synergism was observed on the basis of reductions in minimum inhibitory concentrations (MICs) of these agents[62]. Human beta defensin have been shown to contribute to the defence of the intestine against infection by luminal microsporidia spores[63]. Some of the artificially synthesised α defensin peptide have also shown useful results against various microorganisms[64]. Some reports also suggest that defensin antimicrobial peptides play an important role in host defence, particularly in the oral cavity where there is constant challenge by microorganisms[65]. Some of the recent findings are that in defensin in addition to their antimicrobial and immunomodulatory effects, particularly, HNP-1–HNP-3 possess antiviral and toxin neutralizing properties[66]. Some studies also suggest that HNP-1 along with some other agents like SgIII and DMT-1 are implicated in cell-mediated LDL oxidation for pathogenesis of atherosclerosis[67]. Different studies have also suggested that antimicrobial defensin (HD 5 and HD 6) peptides are important in protection of the host against microbial invasion in states of intestinal inflammation[68].

PRODUCTION OF HUMAN DEFENSIN IN TRANSGENIC PLANTS:

Transgenic Plants are the plants that are produced in the lab using r-DNA technology in order to create plants with special characteristics by artificial insertion of genes from other species, and sometime entirely from different kingdom[69-72]. The plants that are successfully used for making transgenic are rice, corn, tobacco, and soybean. ***Agrobacterium tumefaciens*** is a plant pathogenic bacteria that causes crown gall disease in dicotyledonous plants. The disease is characterized by a tumerous growth on the infected plant. Molecular studies of the disease have revealed that crown gall tumour develops as a result of genetic transformation of the plant by the bacterium. The bacteria make a heritable change in the DNA of the plant and change results in the formation of tumour by the plant. Thus it is an example of naturally occurring genetic engineering and if we can clone our protein/peptides of Interest in the Ti Plasmid i.e. Tumour Inducing plasmid of *A. tumefaciens* and genetically transform the selected plant for example tobacco, the tobacco plants will start expressing the protein/peptide of our interest. **Significance of Using *Nicotiana tabaccum* as Transgenic Plant:** low cost fully functional recombinant proteins have been reported.e.g. relaxin does not serve as a host for human pathogen non food, non feed crop, it does not pose any danger of contamination. Basic peptides and protein can easily be extracted from acidic part. Various antimicrobial peptides and proteins that have been started to be cloned and expressed in the transgenic plants are vaccines (Hepatitis B & Rabies), erythropoietin, human growth hormone, interferons, and various interleukins[73-76]. First Genetically engineered tobacco plants with tolerance by expressing genes encoding for insecticidal proteins from *Bacillus thuringiensis* was reported for the first time in 1987. Thereafter many transgenic plants have been developed by laboratory methods, for example in 1994 Flavr Savv tomato which was the first modern transgenic crop approved for sale in the USA with the advantage of longer shelf life[77]. In 1995 Margherita Zanetti et al have produced cathelicidins **antimicrobial** peptides in transgenic tobacco. A defensin like peptide was produced by Cary et al in the year 2000 in Nicotiana plant. In 2002 Carla M et al cloned and expressed *SMAP-29*- a mammalian antimicrobial peptide of innate immunity[78]. Chenming Zhang in 2005 had successfully produced large quantity of recombinant protein, prorelaxin in transgenic *Nicotiana* plant[79]. In 2006 Gloria Arenas and Sergio H. Marshall have shown that a defensin like Antimicobial Peptide from *Mytilus edulis- chilensis* was cloned into a binary vector and successfully used to transfer in *Nicotiana tobaccum L.* The resulting plant is showing protective effect from a bacterial pathogen *Pseudomonas syringae pv. Syringae*[80].

CONCLUSION

Thus the transgenic plants for production of therapeutic peptides are promising tool for large scale production of this peptide. Further production of defensin like peptide in transgenic plants will also increase immunity towards various biotic and abiotic stresses of plants. The plants will become more resistible for microbial infections. Since human defensin has role in increasing the immunity of the humans therefore plants with defensin expression in it will surely increase the immunity of the individuals who will take it. The purified defensins can also be used as a potential target to be developed as a drug.

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