

## Defensin inspired peptides as novel therapeutic agents

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### Introduction

Antimicrobial peptides are important components of the innate immune response common to invertebrates, vertebrates and plants. Throughout evolution these peptides have retained their antimicrobial potency and are thought to have arisen from multiple, independent sources, possibly through convergent evolution (1). These small diverse peptides provide a rapid first line of response against infection and are generally less than 10 kDa in size, contain 12-60 amino acids and have a cationic nature.

The defensins are diverse members of a large family of cationic host defense peptides, widely distributed throughout the plants and animal kingdoms. These cysteine-rich peptides vary in their length, the spacing of their cysteine residues and their disulfide connectivities (2). Mammalian defensins are generally small (3-6kDa), cationic peptides which have only six conserved cysteines and were originally isolated from rabbit neutrophils (3).

Mammalian defensins can be classified into three subfamilies based on the arrangement of the canonical six cysteine motif and the disulfide bridges that stabilise the  $\beta$ -sheet structure. The defensins consist of the originally isolated  $\alpha$ -defensins, the  $\beta$ -defensins and the more recently identified  $\theta$ -defensins (4). The connectivities of the six cysteine mammalian  $\alpha$ -defensins are C<sup>I</sup>-C<sup>VI</sup>, C<sup>II</sup>-C<sup>IV</sup> and C<sup>III</sup>-C<sup>V</sup> with the  $\beta$ -defensins reported to have a C<sup>I</sup>-C<sup>V</sup>, C<sup>II</sup>-C<sup>IV</sup> and C<sup>III</sup>-C<sup>VI</sup>.

Defb14 the murine orthologue of human  $\beta$ -defensin 3, was made synthetically and studied for both its antimicrobial and chemoattractant properties. Defb14 displayed antimicrobial activity against both Gram positive and negative organisms (*P.aeruginosa* MBC value 1.5  $\mu$ g/ml, *S.aureus* MBC value 3.13  $\mu$ g/ml). Chemotactic activity was observed with HEK293 CCR6 expressing cells at an optimal concentration of 100ng/ml.

Within this study a library of peptides based on a one cysteine analogue of Defb14 were synthesised and analysed for both antimicrobial and chemoattractant properties. Defb141c whereby all the canonical cysteine residues were replaced with alanine residues with the exception of cysteine V was synthesised. The antimicrobial activity profile of Defb141c was similar to that of the original Defb14. Interestingly Defb141c displayed chemotactic properties against HEK293 cells expressing CCR6 comparable to the original Defb14 even though the canonical cysteine motif had been eliminated.

Seven defensin inspired peptides (Dips) were subsequently synthesised displaying differences in net charge, length and hydrophobicity. Both antimicrobial and chemotactic analyses were performed. Antimicrobial activity was discovered in three defensin inspired peptides (Dip1, Dip4 and Dip5), whereby the peptide sequence was located at the N-terminus region. Dip 1 gave a similar antimicrobial profile to that of the full length Defb14 and Defb141c. Interestingly Dips 1, 2 and 3 displayed no chemoattractant properties against HEK293 CCR6 expressing cells unlike that of the full length peptides Defb14 and Defb141c.

This study shows that the murine orthologue of human  $\beta$ -defensin 3 displays potent antimicrobial activity. On designing synthetic peptides based on a one cysteine analogue we can reveal that smaller fragments display comparable activity with that of the parent molecule against a spectrum of both Gram positive and negative organisms. Further analysis reveals that this activity can be attributed to the region associated with the N-terminal sequence. Interestingly the chemoattractant properties of these peptide library fragments are diminished. The importance of the potent antimicrobial activity and the lack of the chemoattractant properties highlight these peptides as ideal candidates for potential therapeutic agents.

### References

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