Anti-tumour immune responses to citrullinated enolase

Katherine Cook1, Ian Daniels1, Victoria Brentville1, Rachael Metheringham1, Wei Xue1, Peter Symonds1, Tracy Pitt1, Mohamed Gijon1 and Lindy Durrant (lindy.durrant@nottingham.ac.uk) 1Scancell Ltd, Nottingham UK, 2University of Nottingham, Nottingham UK

Introduction

Citrullination
Citrullination is a posttranslational modification which occurs as a result of cellular stress and leads to the generation of neoantigens. In rheumatoid arthritis this process generates potent immune responses. Our research suggests that the citrullination of peptides in rapidly growing tumour cells may be exploited to generate specific CD4 responses which lead to tumour rejection.

Enolase
Alpha enolase is a glycolytic protein which is upregulated in many cancers and is known to undergo citrullination. Screening of Enolase peptides identified a citrullinated (cit) peptide which induces an immune response that does not cross react with the wild type (wt) peptide. Responses have been screened against both the human (hu) and mouse (mo) peptide sequences.

This study aimed to determine whether responses to a citrullinated Enolase peptide (Enolase 241-259cit) could have an anti-tumour effect

2. Anti Tumour effect

- B16 F1 and LLC2 Lewis lung carcinoma cell lines were transfected with human HLA-DR4.
- Cell lines were injected s.c. into HLA-DR4 mice at day 1.
- Peptide immunisations were given at day 3. Tumour size and survival was monitored for each mouse

B16 F1 DR4 cell line
Mice immunised with Enolase 241-259cit peptide 3 days after challenge with B16DR4 tumour cells showed increased survival and decreased tumour volume at day 17 post implant

B16 F1 MHCII KO IFNγ-inducible DR4 cell line
Survival advantage was preserved when mice were challenged with a B16 DR4 cell line with murine MHCII knocked out and an IFNγ inducible human HLA-DR4 inserted.

LLC2 DR4 cell line
Mice immunised with Enolase 241-259cit 3 days after challenge with LLC2DR4 but not LLC tumour cells showed increased survival overall unimmunised control mice

Immunisation confers a potent anti-tumour response in vivo

3. Human responses

- PBMCs were isolated from healthy donors and stimulated in vitro with Enolase 241cit or 241wt peptides.
- Thymidine proliferation assays were used to determine proliferation at day 4, 7 and 10.
- Responses in donor 4 were further examined by CFSE proliferation assays and cytokine production was assessed by luminex

Healthy donors
- Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors and stimulated with cit or wt peptides.
- Thymidine proliferation assays showed a number of donors had citrullination specific responses. The time point and extent of responses varied between donors.

Healthy donors have the ability to generate a cit-specific CD4 response to Enolase 241 citpeptide after in vitro stimulation

Donor 4
A proliferation assay on donor 4 showed a CFSE™ CD4+ population specific to the Enolase 241cit stimulated sample

Luminex analysis of supernatants from donor 4 PBMCs showed cit-specific production of IFNγ, IL-17, IL-2, Grb and TNFα

Conclusions

- Transgenic mice expressing human HLA-DR4 or DP4 show a strong response to immunisation with Enolase 241cit peptide
- The immune responses is MHC II mediated and specific to the citrullinated peptides.
- Immunisation with Enolase 241cit peptide was associated with increased survival in anti-tumour experiments
- Healthy donor PBMCs showed responses to Enolase 241cit peptide after in vitro cultures
- Proliferation responses were shown to be CD4 specific and associated with production of multi-cytokines

These results suggest that immunisation with Enolase 241cit peptide may induce citrulline-specific responses which confer an anti-tumour immunity

1Scancell Ltd, Nottingham UK, 2University of Nottingham, Nottingham UK