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**CITRULLINATED GLUCOSE-REGULATED PROTEIN 78 IS A NOVEL CANDIDATE TARGET FOR CANCER IMMUNOTHERAPY**

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**Background** Post translational modification of proteins produces altered epitopes and can play a significant role in immune recognition. Citrullination is the modification of the positively charged arginine amino acid to a neutral charged citrulline residue. This modification is mediated by PAD enzymes and is increased during cellular stress (autophagy). Citrullination results in altered epitopes that can be presented upon MHC class II molecules for recognition by CD4 T cells. Citrullination also occurs in tumour cells as a result of continuous environmental stresses and increased autophagy. We have shown in animal models that the efficient stimulation of citrullinated epitope specific CD4 T cells results in dramatic elimination or regression of tumours. The ER chaperone glucose-regulated protein 78 (GRP78) is required for stress-induced autophagy and is directly linked to autophagosome formation. GRP78 is known to be highly expressed by many tumour types. In this study we investigated the potential of targeting citrullinated GRP78 for cancer therapy.

**Methods** *In vivo* experiments were performed with HLA-transgenic mice under an approved home office licence. Mice were immunised with citrullinated peptides in combination with CpG/MPLA adjuvant. Immune responses were determined using IFN $\gamma$  ELISpot. Anti-tumour studies were carried out by implanting HLA-matched mouse tumour cells subcutaneously and immunising as above. Mass spectrometry analysis was performed to assess peptides presented on tumour cells. Blood samples from healthy individuals were obtained under ethical approval from the University of Nottingham. PBMC responses to citrullinated peptide were assessed using flow cytometry and proliferation assays.

**Results** Five peptides were selected for screening in HLA-transgenic mouse models. One citrullinated GRP78 peptide was identified that gives a CD4 T cell response that is restricted through the HLA DP\*0401 and HLA-DR\*0101 alleles. In addition, this peptide is detected by mass spectrometry in B16 melanoma grown *in vivo*. Anti-tumour studies demonstrated that the citrulline modification-specific CD4 response to this epitope mediates efficient therapy of established B16 melanoma tumours ( $p < 0.0001$ ) in a HLA-transgenic HHDII/DP4 mouse model. Finally, the existence of a repertoire of responses to the citrullinated GRP78 peptide in healthy individuals has been demonstrated with 13/17 (76%) of healthy individuals showing a response to the peptide ( $p = 0.0023$ ).

**Conclusions** Together this data leads us to propose that citrullinated GRP78 is a candidate tumour antigen and that vaccination against citrullinated GRP78 may provide a promising approach for future tumour therapy.

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