Mol Biotechnol (2010) 46:209
DOI 10.1007/s12033-010-9317-y

LETTER

Response to the Letter of Dr. Gabriele Multhoff

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Published online: 28 July 2010

Dear Editor,

In their letter of response to our publication, Dr. Multhoff and Dr. Pockley indicate that the epitope of the monoclonal antibody cmHsp70.1 [1] also described as clone C92F3B1 [2] is located within the 14 aa sequence TKDNNLLGRFELSG (TKD peptide) of Hsp70 (aa 450–463), supported by data from SPOT analysis using 14-mer peptides derived from Hsp70, in which the chimeric and humanized version of cmHsp70.1 revealed an identical binding pattern as cmHsp70.1. Of note, this finding further demonstrates that the two antibodies generated in our laboratory, including the humanized version, retained the antigen reactivity of the parental antibody. However, in our study we found that strong binding of cmHsp70.1 in immunoblotting experiments was observed for a region spanning aa 473–504, which is located in the three-dimensional structure in close proximity to a β-strand containing the TKD sequence, but not with fragments containing the TKD sequence and lacking aa 473–504 or parts of it. Thus, our finding strongly indicates that aa 473–504 are determinants of the main epitope. This does not exclude that the TKD sequence also contributes to antigen binding and specificity, as stated in the discussion, although it is surprising that an antibody raised by immunization with a peptide shows a preferred binding to an adjacent region. Dr. Multhoff and Dr. Pockley do not provide any data showing that the region identified in our study is not recognized by cmHsp70.1 and we are not aware of any published experiments, e.g. competition ELISA, indicating that the TKD peptide represents the entire or main epitope. A peptide SPOT analysis does not allow the analysis of larger regions (as performed in our study with recombinant fragments) and does not give a clear indication about affinity. Therefore, an epitope constituted by 30 or more amino acids will be easily missed by peptide scanning. Thus, in our opinion our findings are not in contradiction to the information provided by Dr. Multhoff and Dr. Pockley.

References

1. Multhoff, G. (2007). Heat shock protein 70 (Hsp70): Membrane location, export and immunological relevance. *Methods, 43*, 229–237.
2. Gehrmann, M., Brunner, M., Pfister, K., Reichle, A., Kremmer, E., & Multhoff, G. (2004). Differential up-regulation of cytosolic and membrane-bound heat shock protein 70 in tumor cells by anti-inflammatory drugs. *Clinical Cancer Research, 10*, 3354–3364.

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