

Clarification of the Functional Significance of Human Folate-binding Protein- α , Peptide 191-199, based on a Correct GenBank Sequence and on Other FBP (191-199) Sequences

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Abstract. *It has been brought to our attention that one of the folate binding protein (FBP) peptides, which we reported first as antigenic and immunogenic in cancer patients, the FBP 191-199, is "off by one amino acid from the amino acid sequence that is listed in GenBank". We searched the published information on FBP and found that the FBP 191-199, which we reported contains threonine 197 instead of the GenBank tyrosine 197. In addition, we found mutations in the FBP (191-199) in other positions, as well as in the flanking residues which direct processing. The potential significance of these changes for cancer vaccines is discussed. It is highly recommended that future human studies with FBP will analyze both GenBank and published sequences in the literature. The large number of mutations in immunogenic FBP-tumor antigens, reported more recently, should be considered during preclinical testing for vaccine and gene therapy in human cancers.*

The recognition of folate-binding protein (FBP) as a cancer antigen and a natural inducer of T-cell immunity in patients with cancer is expected to lead to novel treatments for ovarian cancer, for which little progress has been made in improving overall survival rates during the past 15 years.

It has been brought to our attention, in a copy of an e-mail addressed to a third party, that one of the FBP peptides used and reported by us is actually "off by one amino acid" from the amino acid sequence that is listed in GenBank.

Our peptides were selected in the pre-Internet era (1991-1993), before submission of sequence data to GenBank became routine. However, the sequence codes and dates of

synthesis for peptides we have used are recorded by the Synthetic Antigen Laboratory at M.D. Anderson Cancer Center and can be accessed by contacting Dr. Martin Campbell of the Department of Molecular Pathology at M.D. Anderson. Re-analysis of all of our FBP sequences revealed that the sequence of one of the four FBP peptides we used, E39, was different from the wild-type FBP sequence listed in GenBank. The difference is probably due to mistranslation of the one-letter code for tyrosine (Y) in our sequence to that of threonine (T).

Recent reports have indicated the existence of mutations in FBP, particularly in FBP peptide (191-199), at several positions (1). The first three amino acids of FBP peptide (191-199) in particular are mutated extensively (2) and substitution of a Y at H195 has been found in some ovarian cancer cells (3).

Whether the sequence of the peptide extracted by us in 1991-1993 represents a clone or is the result of a typographical error is unclear. At that time, sequences were typed manually. All of the results related to FBPs published (4-6) were obtained with the peptide E39 (EIWTHSTKV), and not the sequence in GenBank (EIWTHSYKV). There are no typos in the sequence of the other immunogenic FBP peptide, E41, which was recently confirmed (4).

Our results indicate that the variant FBP (191-199) peptide (EIWTHSTKV), which is not naturally processed, is recognized by and immunogenic for T cells that recognize the natural FBP peptide presented by HLA-A2. Our results suggest, but do not directly demonstrate, that the "natural" FBP (191-199) (GenBank EIWTHSYKV) is a natural immunogen in women with ovarian cancer. Although we abandoned our studies of the immunogenicity of FBP peptides in 1999 because of lack of funding, others have since shown the existence of several mutations in FBP (191-199). For example, the N-terminal amino acids E191, I192, and T194 are mutated in FBP from various human sources (2). Changes are also present at the $N_{(-6)}$ to $N_{(-8)}$ and $C_{(+10)}$ positions. Such changes would be expected to affect the yield of FBP processing, peptide presentation, and tumor recognition by cytotoxic T-lymphocytes.

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In light of these published results, our findings should be re-interpreted to indicate that immunity to E39 either may be induced *de novo* by a mutated cancer antigen peptide or may include cross-recognition of E39 by T-cells that had previously been induced by natural variants and the GenBank peptide. It remains to be discovered which peptides are present in health and disease, the significance of the mutations, and how E39 functions in this process.

E39, the wild-type peptide, and all other variants reported so far all share a hydroxyl-free group but have different core sequences. Our reasoning is that if the wild-type peptide (and its variants) are immunogenic, then E39 may transmit an attenuated signal that could prevent apoptosis by overstimulation. The converse – that other peptides are weak and E39 is strong – is also a possibility. The finding that FBP peptide in some tissues contains the mutation Y197 -> F further strengthens the hypothesis that the hydroxyl side chain is immunogenic in cancer (2).

We are pleasantly surprised at the renewed interest in our 10-year-old findings, which prompted us to perform an extensive analysis of the current literature. We hope that our clarification of existing amino acid substitutions in FBPs will be useful in future studies of FBP in immunotherapy and the immunogenetics of ovarian cancer progression.

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