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Tech-Trends Application Note Volume 4, Series 5

Characterization of the Human Folate Receptor Alpha Via Novel Antibody-Based Probes

ABSTRACT: Folate receptor alpha (FRA) is a cell surface protein whose aberrant expression in malignant cells has resulted in its pursuit as a therapeutic target and marker for diagnosis of cancer. The development of immune-based reagents that can reproducibly detect FRA from patient tissue processed by varying methods has been difficult due to the complex post-translational structure of the protein whereby most rea-gents developed to date are highly structure-sensitive and have resulted in equivocal expression results across indep-endent studies. The aim of the present study was to generate novel monoclonal antibodies (mAbs) using modified full length FRA protein as immunogen in order to develop a panel of mAbs to various, non-overlapping epitopes that may serve as diagnostic reagents able to robustly detect FRA-positive disease. Here we report the development of a panel of FRA-specific mAbs that are able to specifically detect FRA using an array of diagnostic platforms and methods. In addition, the methods used to develop these mAbs and their diverse binding properties provide additional information on the three dimensional structure of FRA in its native cell surface configuration.

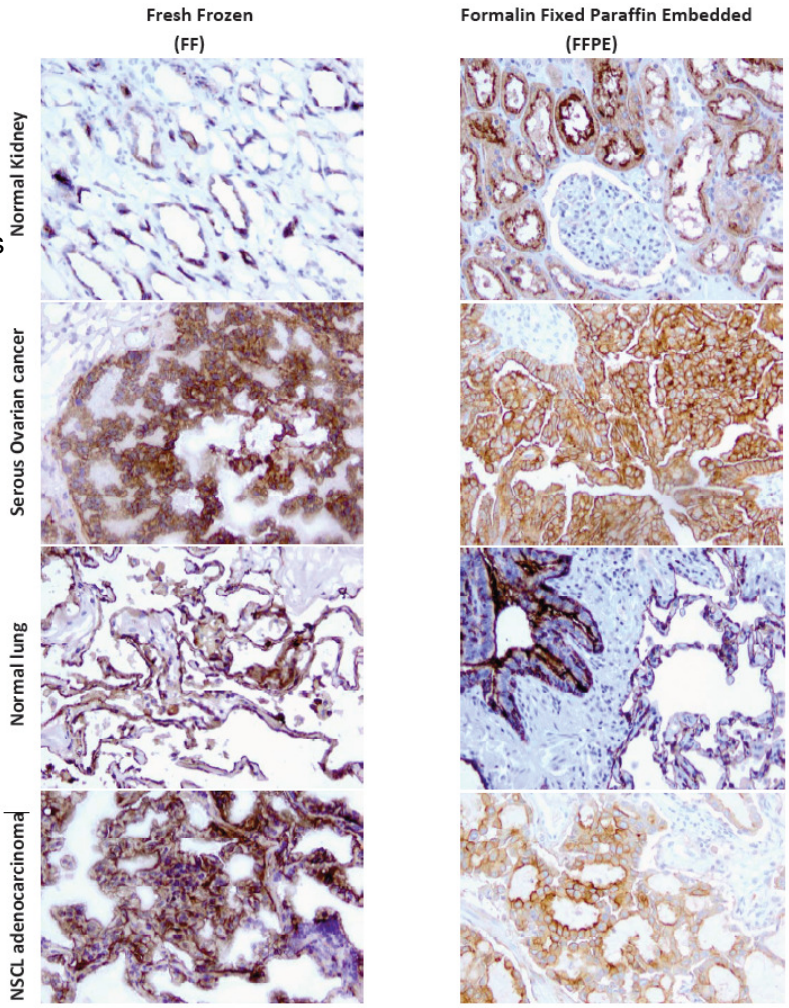

Figure 3: IHC of mAbs on human tissues. mAbs were used to assess the ability to detect FRA in tissues prepared via FFPE or FF methods. Shown is a representative analysis of anti-FRA mAbs developed here that demonstrate specific staining of FRA positive tissues prepared using various methods. mAb 26B3 showed the most robust staining for FRA in IHC and is presented above. Shown is the ability of 26B3 to detect FRA in normal tissues and previously identified FRA-positive cancers (serous ovarian cancer and NSCLC adenocarcinoma) prepared using formalin fixed paraffin embedded (FFPE, right column) and fresh frozen (FF, left column) tissue. As shown, mAb 26B3 is able to robustly recognize antigen in both tissue preparations. Slides shown at 20X magnification.

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METHODS: Five eight-week old female Balb/c mice were immunized with rFRA or dFRA protein as follows. Initial intraperitoneal immunizations administered on day 0 comprised 50µg of rFRA or dFRA prepared as a 1:1 (v:v) mix with complete Freund's adjuvant. Boosts (50µg immunogen mixed 1:1 (v:v) with incomplete Freund's adjuvant were administered intraperitoneally on day 14 and every 21 days thereafter. Test bleeds were collected on day 24 and every 21 days thereafter and analyzed by direct enzyme-linked immunoassay (EIA) against rFRA. Spleens were harvested from animals exhibiting the highest antigen-specific titers and hybridomas were prepared by electrofusion (Hybrimune™ BTX, Holliston, MA) of splenocytes with Sp2/0 Ag14 myeloma cells (ATTC, Rockville, MD). Hybridoma supernatants were rescreened by EIA against rFRA or recombinant mesothelin protein as a negative control, to identify hybridomas producing anti-FRA antibodies. Selected parental cell lines were then sub-cloned by limiting dilution and re-assayed against rFRA by EIA.

RESULTS:

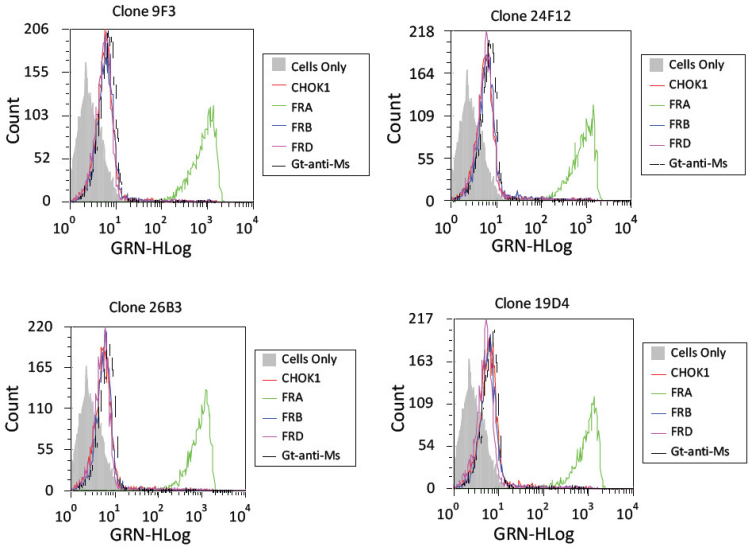

Figure 1: Fluorescence Activated Cell Sorting (FACS). Antibodies were tested for their ability to detect FRA on recombinant CHO cells expressing human FRA, FRB or FRD. Twenty of the 69 mAbs developed here were able to specifically recognize FRA and not the other folate receptor orthologs or negative controls. Shown is a representative analysis of lead antibodies and their robust FRA-specific binding. We then tested the ability of these antibodies to cross react to epitopes or with the previously reported anti-FRA mAb MORAb-003 which is being pursued in clinical development for the treatment of ovarian cancer [20]. Very few mAbs were found to compete for binding. Of particular note was the cross reactivity of 24F12 with 26B3 and 19D4 with MORAb-003 (not shown).

Table 2: Summary Characteristics of Selected mAbs

Antibody	Immunogen Used to Develop mAb	IgG isotype	IHC Reactivity FFPE (+/-)	FACS Analysis+ on FR Expressing Cell Types			Reduced WB Reactivity (+/-)
				FRA	FRB	FRD	
9F3	rFRA	IgG2a	(-)	760	6	5	(-)
24F12	dFRA	IgG1	NT*	777	6	6	(-)
26B3	dFRA	IgG1	(+)	854	6	6	(-)
19D4	dFRA	IgG2a	(+)	1130	NT	NT	(-)

*NT = Not Tested; +values represent fluorescence intensity
rFRA = full length recombinant human folate receptor alpha
dFRA = full length denatured, reduced and alkylated human folate receptor alpha

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