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Folate Receptor alpha is Frequently Expressed in Triple Negative Breast Cancers

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Background

Vitamin B9 (folic acid and folate) is essential to numerous bodily functions. The human body needs folate to synthesize, repair, and methylate DNA as well as to act as a cofactor in certain biological reactions. Folate receptor alpha (FRα) is a membrane-attached protein that facilitates transport of folate, and has been found to be over-expressed in several cancers, including lung, ovary and breast cancers. This finding has led to the development of anti-cancer drugs that target the folic acid receptor.

Studies have shown that over-expression of FRα in breast cancer is strongly correlated with early recurrence and decreased median survival; therefore, FRα has emerged as a potentially promising therapeutic target in breast cancer. A humanized monoclonal antibody to FRα (Farletuzumab) has been developed and may be an attractive treatment strategy either alone or combined with chemotherapy. Therefore, an antibody suitable for immunohistochemistry (IHC) targeting FRα may have significant value in the future, particularly in identifying patients appropriate for treatment with a folate targeted therapy.

Design

A new, highly specific monoclonal mouse anti-FRα antibody, [26B3.F2] (Biocare Medical), suitable for IHC, has been developed and characterized on formalin-fixed paraffin-embedded tissue. Tissues on glass slides were pretreated with an antigen retrieval solution using a modified citrate buffer reagent heated at 95°C for 40 minutes. The FRα antibody was optimally titered and then applied to 67 cases of breast cancer, followed by a micro-polymer HRP detection system (DAB). Only membrane staining for FRα was considered positive, with a cut-off point of 10% of tumor cells staining. All 67 cases were also stained for ER, PR and Her2 status and results were tabulated. Only 3+ Her2 scores were counted as positive in breast cancers, and ER and PR positive cut-off points were deemed positive if >1% of tumor cells were stained.

Results

In normal breast, FRα is expressed in the cytoplasm in normal duct (Figure 1). In most cases of ER and PR positive infiltrating ductal carcinomas, FRα was negative (Table 1). Of the 67 cases of invasive ductal carcinomas stained with clone 26B3.F2, 20 cases were positive for FRα expression. Significantly, a higher incidence of expression of FRα was observed in ER-/PR- patients, independent of Her2 status (Table 1, p=0.003). Only 14.8% (4/27) cases positive for ER and PR were positive for FRα (Table 1, Figure 2); whereas 40% (16/40) of cases negative for ER and PR expressed FRα. Other cases observed were ER/PR positive and/or negative with the co-expression of Her2 and FRα (Figure 3).

Importantly, half of all triple negative specimens 50% (9/18) were positive for FRα (Figure 4), a significant increase over FRα expression in specimens that were ER/PR and/or Her2 positive (22.4%, 11/49) (Table 2, p=0.04).

Table 1: FRα Expression and ER/PR/Her2 Status

	ER+/PR+ Her2+/-	ER-/PR- Her2+/-
FRα **+**	4	16
FRα **-**	23	24
% FRα **+**	14.8%	40.0%

Table 2: FRα Expression in Triple Negative Breast Cancers

	ER+/PR+ and/or Her2+	*ER-/PR- Her2-
FRα +	11	9
FRα -	38	9
% FRα +	22.4%	50.0%

*Triple Negative

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Discussion

Intrigued by the apparent tumor specificity of FRα in lung cancers, we studied its expression in 67 cases of breast cancer. In our study, triple negative breast cancers (TNBC) expressed FRα in 50% of cases. This was a significant increase in TNBC compared to ER/PR and Her2 positive cases. Unless diagnosed early, TNBC have a reduced three-year relapse-free survival compared to women with non-triple-negative tumors. TNBC is typically treated with a combination of therapies such as surgery, radiation therapy, and chemotherapy; however, there are no personalized medicine options for TNBC patients, as found for ER/PR and Her2 positive patients.

Compared to other breast cancers, TNBC tends to grow faster and is less likely to be seen on an annual mammogram; and, it is more likely to spread to other parts of the body earlier. Studies have shown that FRα overexpression in breast cancers is associated with very poor clinical outcome. Although folate-receptor targeted cancer therapies currently in human clinical trials have not yet been tested in breast cancers, their use may be a promising new strategy in treatment for TNBC.

In this study, an increase in expression of FRα was also observed in Her2 positive cases (22.4%). This could also represent a subtype of Her2 positive cases that may not respond to treatment.

Finally, there was a small subset of ER/PR positive cases that expressed FRα, and this could also represent a high risk factor not seen before.

Conclusion

FRα expression has been identified in 30% (20/67) of breast cancers, with an increased incidence in ER/PR negative cases. Significantly, 50% (9/18) of triple negative breast cancers were positive for FRα expression. Determining positive FRα expression in breast cancers may identify patients that would benefit from an anti-folate targeted therapy, including hormone receptor or Her2 positive patients that have failed to respond to prior treatment; or triple negative patients for whom limited treatment options are available. FRα targeted therapies, alone or in combination with cytotoxic chemotherapeutics, may represent a novel approach to treatment for TNBC.

Figures

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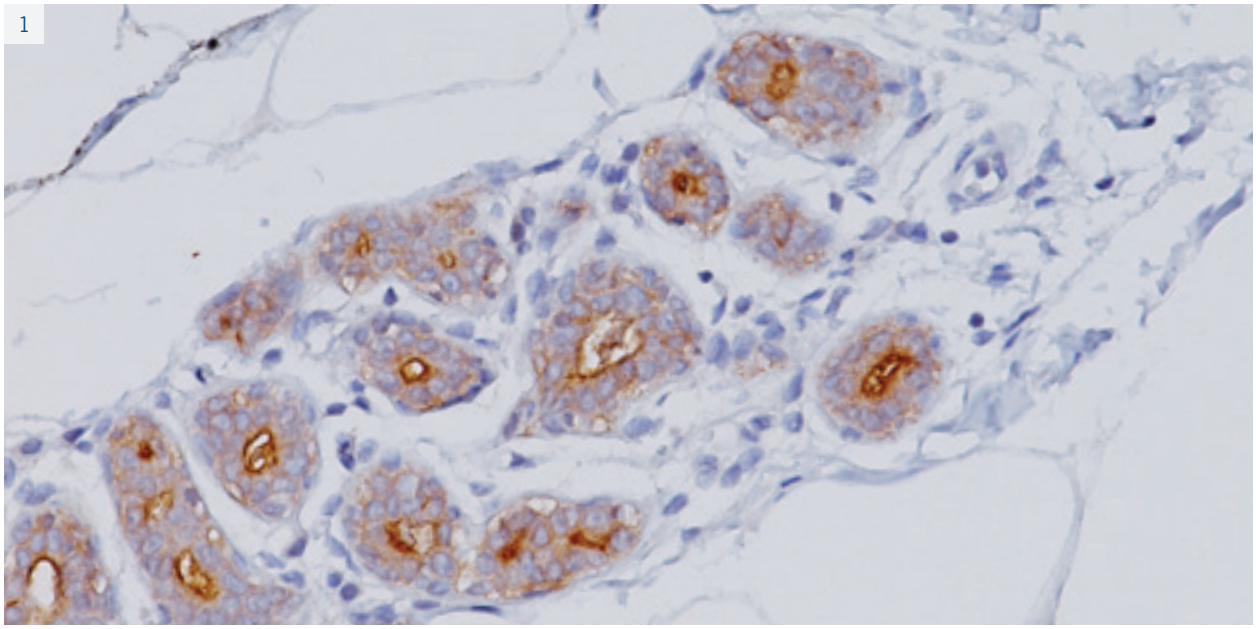
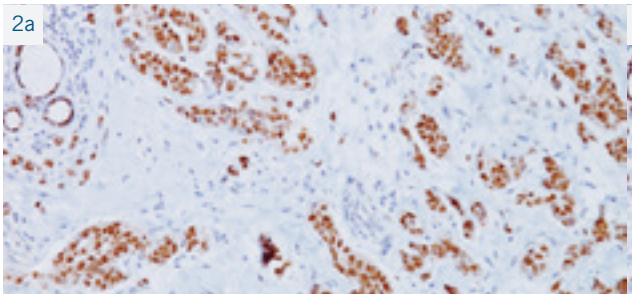
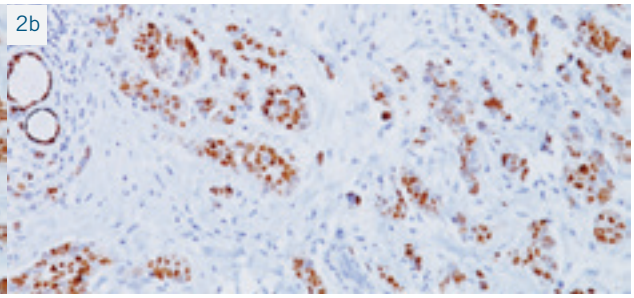


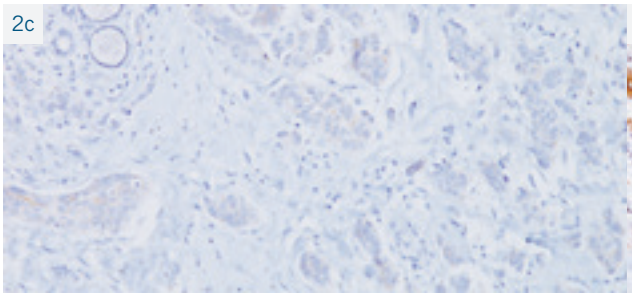
Normal adjacent breast ducts stained with FRα



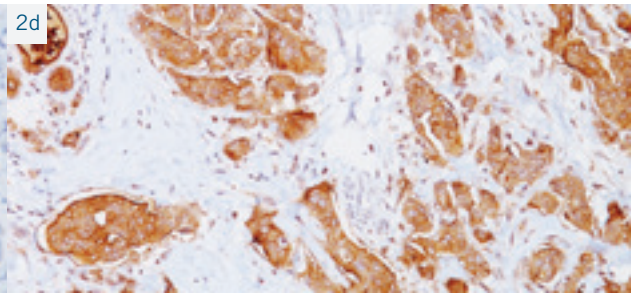
Invasive breast cancer demonstrating ER+



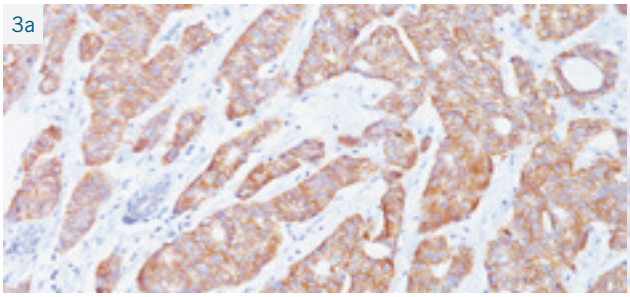
Invasive breast cancer demonstrating PR+



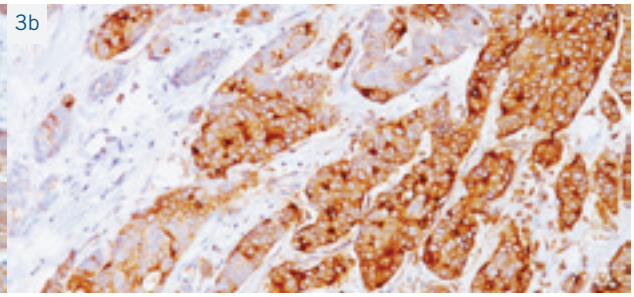
Invasive breast cancer demonstrating Her2-



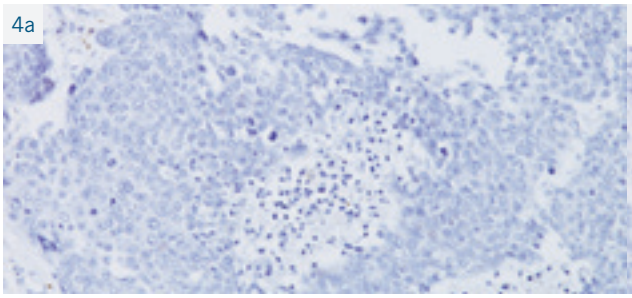
Invasive breast cancer demonstrating FRα+



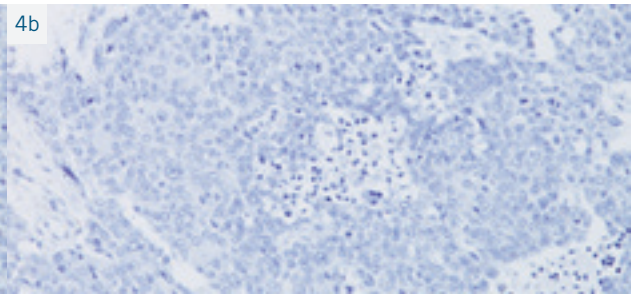
Invasive breast cancer demonstrating Her2+



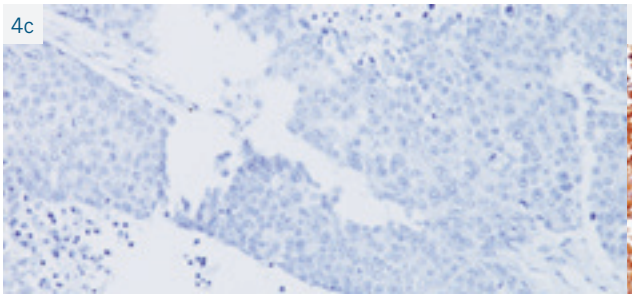
Invasive breast cancer demonstrating FRα+



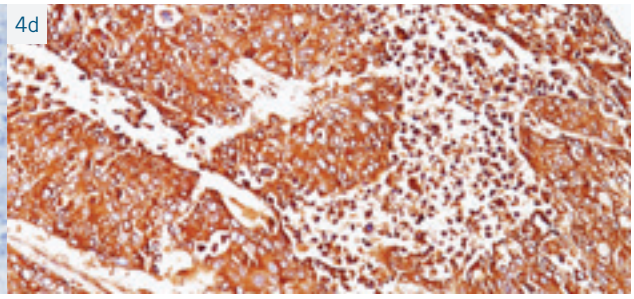
Triple negative breast cancer demonstrating ER-



Triple negative breast cancer demonstrating PR-



Triple negative breast cancer demonstrating Her2-



Triple negative breast cancer demonstrating FRα+

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