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Original Article

Comparative study of immunohistological quantitative analysis of c-erbB-2 oncoprotein in mammary cancer by two different antibodies, a conventional anti- c-erbB-2 oncoprotein antibody (Novocastra Lab. Co.) and HercepTest (Dako Japan Co.) - Could an ordinary anti-c-erbB-2 oncoprotein antibody substitute for HercepTest ? -

Pathology Center

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Objective: C-erbB-2 oncoprotein was cell surface growth factor receptor and applied to the prediction of treatment effect of mammary cancer. Could HercepTest (Dako Japan Co.) be replaced by other conventional primary antibody, like anti-c-erbB-2 oncoprotein antibody (Novocastra Lab. Co.) for an immunohistological examination of c-erbB-2 oncoprotein in mammary cancer.

Study design: A routinely processed formalin-fixed and paraffin-embedded post-operative specimens from 48 cases of mammary adenocarcinoma were stained immunohistologically with HercepTest kit (Dako) and other one derived from Novocastra.

Results: Anti-c-erbB-2 oncoprotein antibody showed fair stainability and gave a significant simple linear regression formula: (the predictive positive value in HercepTest) = 0.88 x (the observed positive value in traditional c-erbB-2 staining) - 0.1.

Conclusion: Anti- c-erbB-2 oncoprotein antibody (Novocastra) could take the place of HercepTest (Dako) in mass-screening of c-erbB-2 oncoprotein of mammary cancer.

Key words: c-erbB-2, anti- c-erbB-2 oncoprotein antibody (Novocastra Lab. Co.), HercepTest (Dako Japan Co.), mammary cancer

Introduction

C-erbB-2 oncoprotein was cell surface growth factor receptor, i.e., epidermal growth factor receptor. An excessive expression of c-erbB-2 oncoprotein was shown in mammary cancer, adenocarcinoma, and transitional cell carcinoma, which was regarded as being a poor prognosis and recently used to predict the efficacy of specific antibody treatment.1) The degree of excessive expression could be decided immunohistologically.

There were several problems to get both a stable dyeing technique and an objective judgment of staining intensity. In order to standardize this judging method, the immunohistological overexpression in mammary cancer was recommended to determine by the standardized commercial reagent kit, i.e., HercepTest (Dako Japan Co.). This kit reagent had been diluted appropriately at the time of acquisition and, furthermore, even the guidelines of both a dyeing manual and a figure dyeing for determination was prescribed in it. Although this kit was clinically convenient to obtain a steady dyeing result easily, there remained three shortcomings. This kit antibody had been diluted so abundantly that the staining ability fell off rapidly and could not be preserved for a long time. Furthermore, it cost 5400 yen to use only one test except any other cost and a cost rate of material reached to 57%, which made examiners feel expensive. Also, the discrimination of a staining strength of 2+ from that of 1+ remained difficult, whose discriminating point was very important for a prediction of treatment effect (Table 1, Fig. 1).

Table 1. Attached determination standard in Hercep Test (Dako Japan Co.)

excessive expression of c-erbB-2 oncoprotein	staining score	staining pattern	
		positive cell rate (%)	staining strength
-	0	<10	
-	1+	10≦	weak
+	2+	10≦	moderate
+	3+	10≦	severe

Conventionally, we have used anti- c-erbB-2 oncoprotein antibody (Novocastra Lab. Co.) as the first antibody to confirm c-erbB-2 oncoprotein expression in

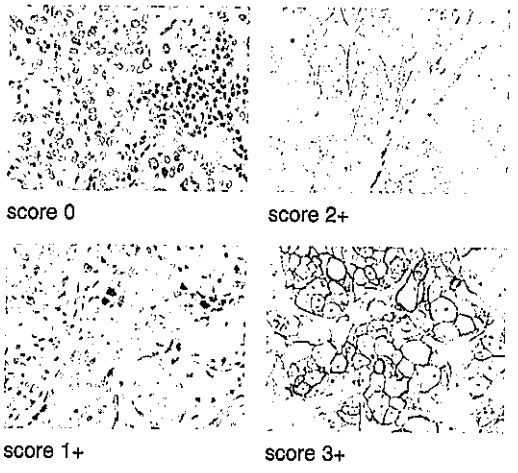

Fig 1. Attached photograph for determination of HercepTest (Dako)

tumors. It was possible to preserve its stainability for a long time and to reduce a cost of material markedly. Therefore, if a steady stainability could be gotten by this usual anti- c-erbB-2 oncoprotein antibody, this inexpensive and durable antibody could substitute for the expensive and fragile HercepTest as a screening test for overexpression of c-erbB-2 oncoprotein. We examined by this antibody whether to get the same stainability as HercepTest.

Material and method

Immunohistologic analysis was done in 48 cases of mammary adenocarcinoma from the list of Pathology Center of JA Niigata Pref. Public Welfare Association in 2002. These post-operative specimens had been fixed by neutral formalin during less than 4 days and embedded in paraffin. The predominant pathology consisted of (1) intraductal early adenocarcinoma: 2 cases, (2) invasive adenocarcinomas: 1 case of apocrine carcinoma, 1 case of lobular carcinoma, 1 case of mucinous carcinoma, 12 cases of papillo-tubular carcinoma, and 21 cases of scirrhous carcinoma (Table 2).

Immunostaining technique had been already mentioned.1) The examined first antibodies consisted of both anti- c-erbB-2 oncoprotein antibody (Novocastra Lab. Co.) and HercepTest (Dako Japan Co.). The determination of positive dyeing strength depended on the determination photograph in the HercepTest (Table 1, Fig. 1).

A statistical analysis was done based on Exel analysis tool (Microsoft, USA): Correlation coefficient, simple linear regression, t-test (non-parametric test).

Result

The positive rate for anti- c-erbB-2 oncoprotein antibody and HercepTest was 69% and 49%, respectively.

Table 2. Caes, staining results and main histology

histology number	c-erbB-2 positivity		main histology
	c-erbB-2 (Novocastra Lab. Co.)	HercepTest (Dako Japan Co.)	
11528	0	0	sc
11583	0	0	st
11683	2	3	sc
11707	0	0	sc
12281	2	2.5	pt, co, ap
12463	3	3	sc
12592	1	1	sc
12648	3	1.5	sc
12673	0	0	pt
12764	0	0	pt
12825	3	3	pt
12939	0	0	sc
13310	0.5	0	sc
13730	0	0	sc
13841	1	0	sc
13767	0	0	pt
13895	3	3	st
13968	1	1	pt
14220	0	0	st
14215	1	0	st
14221	0	0	st
14302	0	0	ap
14374	0	0	sc
14562	0	0	pt
14567	0.5	0	sc
14568	1.5	1	lo
14750	1	0	in, co
15355	1	1	st
15716	3	3	sc
16371	1	0	sc
16520	1	1	st
16611	0	1	sc
16900	1	3	pt, co
16933	0	0	pt
17357	2	2	sc
17880	1	0	pt
18110	1	0	in
18112	3	3	st
18530	2	1	pt
18656	3	3	sc
19072	3	0	sc
19231	1	0	mu
19343	3	3	pt
19473	1	0	pt, co
19781	3	3	sc
19881	2	1	sc
19959	3	3	sc
20201	0.5	0	st

abbreviation

1 in: intraductal, pt: papillotubular, co: comedo, st: solid-tubular, sc: scirrhous, mu: musinous, lo: lobular, ap: apocrine

2 positivity : 0: negative、1 : mild、2 : medium、3 : severe

The correlation coefficient of the positive dyeing strength between anti- c-erbB-2 oncoprotein antibody and HercepTest reached to 0.82 (0.70-0.88, 95% of a reliability rate). The analysis with simple linear regression brought the following expected formula: (the expected positive dyeing value in HercepTest) = 0.88 x (the observed positive dyeing value in anti- c-erbB-2 oncoprotein antibody) - 0.1 (Fig. 2). There was no significant difference among main pathology.

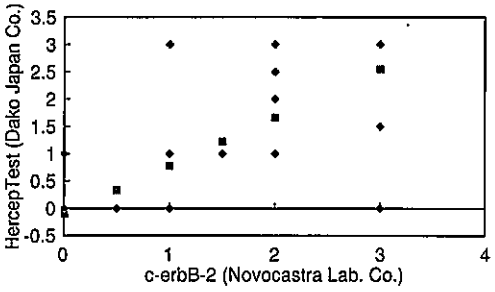

Fig 2. Comparison of staining positivity between c-erbB-2 (Novocastra Lab. Co.) and HercepTest (Dako Japan Co.). black solid diamond: actual positive value in HercepTest, light solid square: statistically-expected positive value in Hercep Test

Discussion

In mammary carcinoma, it was important to detect an overexpression of c-erbB-2 oncoprotein for a prediction of both their prognosis and the effect of specific treatment. For this purpose an immunohistological determination was more practical and convenient than a purely biochemical examination. It became important that the amount of c-erbB-2 oncoprotein could be more accurately measured immunohistologically, and HercepTest (Dako Japan Co.) was recommended to use for this sake. However, a short valid term for stainability and an additional staining procedure of HercepTest brought several complicated problems under the circumstances where a business rationalization and an expense cut were emphasized. We examined the possibility of anti- c-erbB-2 oncoprotein antibody (Novocastra Lab. Co.) substituted for HercepTest and got a statistically reliable positive correlation: correlation coefficient was 0.82 (95% of reliability).

Also, a statistical analysis with simple linear regression revealed the following formula: (the expected dyeing value in HercepTest) = 0.88 x (the observed dyeing value in anti- c-erbB-2 oncoprotein antibody) - 0.1. This equation had an advantage to neglect any delicate judgement of stainability: an immuhistologically threshold, above which the specific treatment was expected, was between the point 1+ and 2+ in HerceptTest, which was delicately different, and ultimately we did not have to consider this ambiguous discrimination in stainability by using anti- c-erbB-2 oncoprotein antibody because all the cases more than the criterion point of 2+ in HerceptTest were simply classified into one group, i.e., the strongest stainability group (3+ in grade on HercepTest reference photograph) (Fig. 2).

A sufficient dyeing training in each institution may be necessary to obtain this steady correlation nature by other corresponding staining reagents substituted for HercepTest. It becomes, furthermore, possible to avoid both a gradual diminution of stainability and an additional staining techniques in the complicated situation requiring many various complicated immunodyeing operations as daily examinations.

Referrence

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和 文 抄 録

原著

2種の抗体、従来型のanti- c-erbB-2 oncoprotein抗体（Novocastra Lab. Co.）とHercepTest (Dako Japan Co.)による、乳癌のc-erbB-2 oncoprotein発現量の免疫組織学的定量分析の比較研究 －通常のanti-c-erbB-2 oncoprotein抗体がHercepTestを代行できるか？－

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目的：C erbB-2 oncoproteinは細胞表面成長ファクター受容体であり、乳癌（乳腺癌）の治療効果の予測に応用されている。乳癌c-erbB-2 oncoproteinの免疫組織学的検査において、従来型のanti-c-erbB-2 oncoprotein抗体（Novocastra社製）は、HercepTest（Dako日本社製）のスクリーニング代用になりうるかを検討した。

方法：乳腺癌で切除された48症例乳腺組織について、中性ホルマリン固定・パラフィン包埋された組織の優勢組織像部分に関して、HercepTestキット（Dako）および他の代用抗体（Novocastra）による免疫染色を実施した。

結果：Anti-c-erbB-2 oncoprotein抗体（Novocastra）使用標本は、満足すべき染色結果であった。また、その染色度を比較して、簡単な相関式を得ることができた：(HercepTest染色における予想score)＝0.88×(Novocastra抗体による実際の染色度score)－0.1。

結論：乳癌c-erbB-2 oncoprotein発現の免疫組織学的マススクリーニング検査において、Anti-c-erbB-2 oncoprotein抗体（Novocastra）がHercepTest（Dako）に取って代わることができると判断された。

キーワード：c-erbB-2、抗c-erbB-2 oncoprotein抗体(Novocastra Lab. Co.)、HercepTest (Dako Japan Co.)、乳癌