Classification of gastric cancer patients based on HLA antigen expression using quantification method III

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We have previously presented results suggesting that HLA antigen status could predict the response of cancer to therapy, and have proposed that rational individualized therapy for cancer patients might possibly be designed by evaluating HLA antigens. In this study, we classified gastric cancer patients into four groups using quantification method III and then developed a simple method of classifying patients to HLA antigen status for easy clinical application.

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Development of parameters that correlate with the response of cancer patients to treatment would allow the more rational and suitable use of anticancer therapy. The human major histocompatibility complex(MHC), also referred to as the human leucocyte antigen(HLA) complex, is located on a small part of chromosome 6, and is by far the most variable part of the genome and initiates immune responses. We have previously presented results suggesting that HLA status could predict the response to anticancer therapy, and have proposed that rational individualized therapy for cancer patients might possibly be designed by evaluating HLA antigens¹⁾.

The survival of gastric cancer patients in Japan appears to be better than in the West²⁾, suggesting that patients may respond differently to anticancer therapy in Japan or that there are different patterns of gastric cancer in Japan and Western countries³⁾.

We know that the HLA antigen status of the Japanese is different from that of Caucasians. Thus, a different response to anticancer therapy combined with different therapeutic strategies may produce distinct outcomes.

In this study, we classified gastric cancer patients using quantification method III, which is one method of multidimensional data analysis^{4,5)} and is equivalent to the correspondence analysis method by Benzécri^{6,7)}. We then developed a simple method of classifying patients according to HLA antigen status for easy clinical application.

Fig. 1 Scattergram of the HLA antigens in 626 patients (quantification method III)

Materials and methods

We first analyzed the data of 626 gastric cancer patients and then assessed 741 patients. We examined HLA A, B, C, DR and DQ loci using the assay. HLA antigens with a frequency > 1% and blank antigens were assessed as follows : A loci-2, 3, 9, 10, 11, 31, 33 : B loci-7, 12, 13, 15, 16, 35, 46, 48, 51, 52, 54, 55, 56, 59, 60, 61 : C loci - w1, w3, w4, w7 : DR loci - 1, 2, 4, 5, 6,

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Table 1 The main HLA antigens in each cluster

b	A3, A33, B12, B13, DR6
c	Bw46, Bw54, Bw55, Bw56, Bw59, Cw1, DR4, DQw4 (B22 was subdivided to Bw54, Bw55, Bw56)
d	B 7, B15, Cw7, DR1

Table 2 The functions dividing patients scattered on 2-dimensional space 1Y and 2Y

Type I	: $^1Y^2 + ^2Y^2 < 0.7$
Type II	: $^2Y < ^1Y/3$ and $^2Y \leq 3 * ^1Y$, except type I
Type III	: $^2Y < -2 * ^1Y$ and $^2Y > 3 * ^1Y$, except type I
Type IV	: $^2Y > ^2Y/3$ and $^2Y \leq -2 * ^1Y$, except type I

Fig. 2 Scattergram of the individual patients

8, 9 : DQ loci - 1, 3, 4 : and A blank, B blank, Cw blank, DR blank, and DQ blank.

Figure 1 shows a scattergram of the HLA antigen status of 626 patients. Cluster a is located in the center,

and the remaining b, c, and d clusters located in each direction ($1X * 2X$). Table 1 shows the HLA antigens in each cluster. Individual patients are scattered in two-dimensional regions that ($1Y * 2Y$) correspond to the

Table 3 The relationship of types I, II, III, and IV to type T(b), T(c), and T(d)

	Type I	Type II	Type III	Type IV	Total
T(b) & N(c) & N(d)	0	77	0	0	77
T(b) & T(c) & N(d)	1	4	0	0	5
T(b) & N(c) & T(d)	0	3	0	4	7
N(b) & T(c) & N(d)	11	0	88	0	99
N(b) & T(c) & T(d)	1	0	6	2	9
N(b) & N(c) & T(d)	15	0	1	74	90
N(b) & N(c) & N(d)	244	18	71	6	339
Total	272	102	166	86	626

note : There were no patients with type T(b) & T(c) & T(d)

Table 4 Cross-tabulation of types I, II, III, and IV with types I', II', III', and IV'

	Type I	Type II	Type III	Type IV	Total
Type I'	244	18	71	6	339(54.2%)
Type II'	1	81	0	0	82(13.1%)
Type III'	12	0	94	2	108(17.3%)
Type IV'	15	3	1	78	97(15.5%)
Total	272 (43.5%)	102 (16.3%)	166 (26.5%)	86 (13.7%)	626(100.0%) (100.0%)

Table 5 Cross-tabulation of types I, II, III, and IV with types I', II', III', and IV' in the 741 patients

	Type I	Type II	Type III	Type IV	Total
Type I'	272	20	96	11	399(53.8%)
Type II'	2	93	0	0	95(12.8%)
Type III'	32	0	93	3	128(17.3%)
Type IV'	8	3	6	102	119(16.1%)
Total	314 (42.4%)	116 (15.7%)	195 (26.3%)	116 (15.7%)	741(100.0%) (100.0%)

Fig. 3 Scattergram of the HLA antigens in 741 patients

clusters of HLA antigens (Fig. 2). We divided the 626 patients into four types (I - IV) according to the functions shown in Table 2. Type II patients are characterized by HLA antigens of cluster b, type III by cluster c and type IV by cluster d. Type I is a common type that does not show any characteristics.

We then defined three other groups. T(b) was a group of responders who had 2 or more HLA antigens of cluster b, while the rest of the patients were N(b). T(c) was a group of responders who had 3 or more HLA antigens of cluster c, and while the rest were N(c). T(d) was a group of responders who had 2 or more HLA antigens of cluster d, while the rest were N(d). The relationship between cluster types and groups obtained by counting the number of HLA antigens is shown in Table 3. A detailed classification of the patients was performed as follows: (T(b) & N(c) & N(d)) or (T(b) & T(c) & N(d)) was classed as type II', (N(b) & T(c) & N(d)) or (N(b) & T(c) & T(d)) was classed as type III', (N(b) & N(c) & T(d)) or (T(b) & N(c) & T(d)) was classed as type IV', and all others were called type I'. Table 4 shows the relationships between types I to IV

and types I' to IV'.

A scattergram of the HLA antigen status of 741 patients is shown in Figure 3, with the results being almost the same as in the previous patients. The relationship between patient types classified by HLA cluster and by the number of HLA antigens is shown in Table 5.

Discussion

We used a method of quantification of response pattern and classified similar HLA antigens into four groups. This method is usually used for multidimensional data analysis, e. g. , in the survey of national character^{8,9)}, and is one method of classifying individuals based on the similarity of their pattern of categorical responses to questions. Using this method, the correspondence of two configurations of the individuals and the categories in multidimensional Euclidian space can be used to make inferences with regard to their categories.

We previously reported on the distribution of HLA antigens in gastric cancer patients¹⁾. The results of the previous and present studies were very similar. However, slight axis rotation and a slightly different location was noted in this study, especially for clusters a and c. Despite this, the structure remained stable and the HLA antigens in each cluster were almost stable. So there was no major difference between these studies. Thus, the classification of HLA antigens by quantification analysis is stable, but patients cannot be classified by a fixed equation. Therefore, a simple new classification based on

the number of HLA antigens was developed to apply clinically. A prospective randomized study using this new classification is now on going to confirm its clinical value in gastric cancer patients.

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Correlation between argyrophilic nucleolar organizer regions and immunostaining for carbohydrate antigen 19-9 in human gastric cancers

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【目的】胃癌における腫瘍組織内CA19-9の免疫組織化学的発現を検討すると同時に、腫瘍生物学的悪性度の一指標であるAgNORs数を測定し、CA19-9染色強度とAgNORs数との相関を検討した。また、臨床病理学的因子別にAgNORs数、CA19-9発現率を求め比較検討した。

【対象と方法】胃癌50症例を対象に、ホルマリン固定後のパラフィン切片を用い癌部の薄切片を作製後、Plotonらの方法に準じ銀染色を行い、腫瘍細胞核200個の総AgNORs数を求め、1核当たりのAgNORs数を求めた。同時に抗CA19-9モノクローナル抗体を用い、SAB法により腫瘍組織内を免疫組織化学的染色した。なお、染色強度は G_0 : negative type, G_1 : apical or focal cytoplasmic type, G_2 : diffuse cytoplasmic type, G_3 : stromal type の4型に分類した。

【結果】(1) 胃癌50例中、早期胃癌、進行胃癌のAgNORs数は 4.99 ± 1.33 , 7.04 ± 2.45 であり、進行胃癌が有意に高値であった。また、CA19-9の発現は33例(66.0%)において認められた。

(2) 染色強度別のAgNORs数は G_0 : 5.19 ± 1.63 ($n=17$), G_1 : 5.78 ± 2.26 ($n=5$), G_2 : 6.82 ± 2.23 ($n=16$), G_3 : 7.76 ± 2.80 ($n=12$) であり、 G_2 , G_3 は G_0 にくらべて有意に高値であった。

(3) 病理学的因子別にみたAgNORs数は肉眼的進行度別にはstage IVはstage I, IIにくらべて有意に高値であり、腹膜播種性転移陽性例、漿膜浸潤陽性例、リンパ節転移陽性例においてAgNORs数は有意に高値であった。

(4) 病理学的因子とCA19-9染色陽性率との関連は有意な相関は認めなかった。

(5) 胃癌50例のAgNORs数の平均値である6.48をcut off pointとして50症例をAgNORs高値群、低値群に2分し、累積生存率を求めたところ、AgNORs高値群の予後が有意に有効であった。

【考察】胃癌における腫瘍組織内CA19-9発現は古くはAtkinsonらの報告があるものの、いまだその臨床病理学的意義については明らかではない。今回、筆者らの症例では33例(66.0%)にその発現を認めたが、病理学的因子との相関は認めなかった。一方、腫瘍増殖能を反映するAgNORs数については進行癌は早期癌にくらべて有意に高値であり、さきの癌組織内CA19-9発現形式別にみれば、diffuse cytoplasmic type, stromal typeにおいてAgNORs数は高値であり、腫瘍増殖能の強度な細胞は機能的にCA19-9産生が亢進している点がかがえた。胃癌において癌進展度の強度例はAgNORs数は高値でありその相関性が認められた。さらに、AgNORs数高値群は明らかに予後不良であり、胃癌における予後規定因子になりうる可能性が示唆された。

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HLA抗原によって胃癌の治療に対する反応が予測でき、HLA抗原を測定することにより、癌患者個人個人に適切な治療ができることをすでに発表している。本研究では、HLA抗原治療効果の関係をみるための、HLA抗原に基づく胃癌患者の分類を、数量化III類を使って行った。さらに臨床の場で適用しやすいようにその簡便な分類法を示した。

数量化III類は林の開発した多次元解析の一つであり、調査対象(患者)の調査項目(HLA抗原)への反応パターンから項目の分類、対象の分類を同時に行うという考え方に基づく方法である。まず、626人のデータから分析し、後に741人のデータについて分析し、その安定性を確認した。

HLA抗原は1%以上の頻度のあるものを取り上げ、次の42抗原についての分析となった。

数量化分析の結果得られた42抗原の付置から、四つのクラスターができることがわかった。bクラスターはA3, A33, B12, B13, DR6: cクラスターはBw46, Bw54, Bw55, Bw56, Bw59, Cw1, DR4, DQw4, Bw54, Bw55, Bw56: dクラスターは, B7, B15, Cw7, DR1 で、残りがaクラスターである。これに対応して得られる患者の付置から患者を四つの型(I型, II型, III型, IV型)に分けることができる。この患者の型はもっているHLA抗原をパターンでみた特徴としてとらえた分類である。

ここで、この特徴を簡便にとらえるために、別の分類を考えた。T(b)はクラスターbのHLA抗原を二つ以上もつ患者でそれ以外がN(b), T(c)はクラスターcのHLA抗原を三つ以上もつ患者でそれ以外がN(c), T(d)はクラスターdのHLA抗原を二つ以上もつ患者でそれ以外がN(d), とした。これらの三つの分類は重複しているので改めてつぎのように四つの型を作成した。すなわち、(T(b)&N(c)&N(d)) or (T(b)&T(c)&N(d))ならばI'型, (N(b)&T(c)&N(d)) or (N(b) & T(c) & T(d))ならばII'型, (N(b) & N(c) & T(d)) or (T(b) & N(c)) & T(d))ならばIII'型, そしてこれ以外のすべてをIV'型とした。I~IVの型分類とI'~IV'の型分類との相関は非常に高いことが示された。

741例のデータに関しても同様の分析を行った結果、42抗原の付置に多少の違いはあるが、クラスターとして安定していることが示された。2種類の群分けの相関関係も同様に安定していた。このことから、データが新しくなるごとに数量化III類の分析を行わなくても、決まったクラスターに属するHLA抗原の数を数えていけば作成できる後述のI'~IV'の群分けは、今後臨床データで治療効果とHLA抗原の関係をみていくうえで有用である。