FLUORESCENCE IN SITU HYBRIDIZATION IN IRANIAN PATIENTS WITH PRIMARY BREAST CANCER

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Abstract — *Breast cancer is presentative of the genetic heterogeneous alterations. In the present investigation, fluorescence in situ hybridization (FISH) was performed on the fresh primary tumours of 6 patients with infilterating ductal carcinoma. DNA-probe for centromere of chromosome 3 (3cen) was applied and in the total analysis, the majority of interphases presented 1 signal (33.6%) which followed by 3 (28.8%), 2 (16.8%) and 4 (13.5%) signals. Only two tomours showed the +presence of more than 6 signals. The presence of 2 signals could be observed in only one tumour (19.6%). In two tumours, 3 signals (33.1%) was considered as the most frequent alteration and followed by more than 6 signals (25.5% and 18.3% respectively), in tumour ID 27, and 3 signals (43.1%) followed by 1 and 4 signals (30.1% and 17.2% respectively) in tumour ID 33. It is concluded that the FISH-technique is able to clarify and diagnose the numerical alterations of chromosome 3 cen in tumour cells of BC patients.*
Acta Medica Iranica 34 (3 & 4): 80-82; 1996

Key words: Fluorescence in situ hybridization (FISH), breast cancer, Iran.

INTRODUCTION

Breast cancer (BC) is considered to be the most frequent cancer in women. The literature risk is found to be 1 in 8 in the northern and western European population (1) and is the second major cause of death in women. In spite of high incidence of BC, absence of clear genetic data, including the cytogenetic changes all reflect the difficulties of procedures performing on the tumours. However, more investigations have been carried out on the basis of flourescence in situ hybridization (2,3,4). This technique was also named interphase molecular cytogenetics which facilitates the study of numerical chromosome changes in the interphase of tumour cells. These data revealed the variation of signals' count with a heterogeneous distribution over the nuclei of chromosomal region (cen). Although there is no consistent involvement of specific chromosome alterations in breast tumours, but aberation of chromosome 3 is found to be one of the common ones (5,6,7,8). Therefore, it is important to investigate and detect the genetic alterations in the primary tumours of BC. The present data is a part of our initial oncogenetic investigation from Iran (9).

MATERIALS AND METHODS

The fresh primary tumours of 6 patients with infilterating ductal carcinoma (IDC) were directly cultured post-surgery and the cell preparations were made (Table 1).

The fluorescence in situ hybridization (FISH) was performed with application of DNA-Probe for centromere of chromosome 3 (3 cen) (2,3). FISH-technique was applied according to the following procedures:

- Slides were denatured with formamide at 75°C, then dehydrated in 70% , 90% and 100% ethanol.
- Hybridization in Hybrisol and biotinylated probe (overnight incubation at 37°C).
- Slides were rinsed in formamide
- Detection and amplification of the signals were performed with Avidin/FITC and goat-anti-Avidin.
- Staining with propidium iodide/DAPI.

The distribution of signals is presented on the basis of signal analysis in 613 cells (Table 1) and lymphocytes were used as quality control.

RESULTS

Table 1 presents tumours distribution according to the pathologic classification and the signals-count in individual and the total of tumours. The number of signals ranged between 1-6 and more than 6 (Fig. 1). In the total analysis, the majority of interphases revealed to present 1 signal (33.6%) followed by 3 signals (27.8%), 2 signals (16.8%) and 4 signals (13.5%). Only 2 tumours (33.3%) showed the presence of more than 6 (up to 10) signals.

Table 1. Distribution of hybridization - signal of DNA - Probe 3 cen in the tumours of 6 breast cancer with infiltrating ductal carcinomas (IDC).

Patients'ID	Percentage of cells with a ginen number of signals							Total number of cells
	1	2	3	4	5	6	>6	
8	32.2% (20)	56.4% (35)	9.6% (6)	1.6% (1)				62
20	58.4% (31)	28.3% (15)	9.4% (5)	3.7% (2)				53
27	12.7% (25)	5.1% (10)	33.1% (65)	25.5% (50)	5.1% (10)		18.3 (36)*	196
28	45.9% (45)	13.2% (13)	30.6% (30)	10.2% (10)				98
29	56.8% (50)	25% (22)	17% (15)			1.1% (1)		88
33	30.1% (35)	6.8% (8)	43.1% (50)	17.2% (20)		1.7% (2)	0.8% (1)**	116
Total	33.6% (206)	16.8% (103)	27.8% (171)	13.5% (83)	1.6% (10)	0.4% (3)	6% (37)	613

* 7-10 signals
** 9 signals

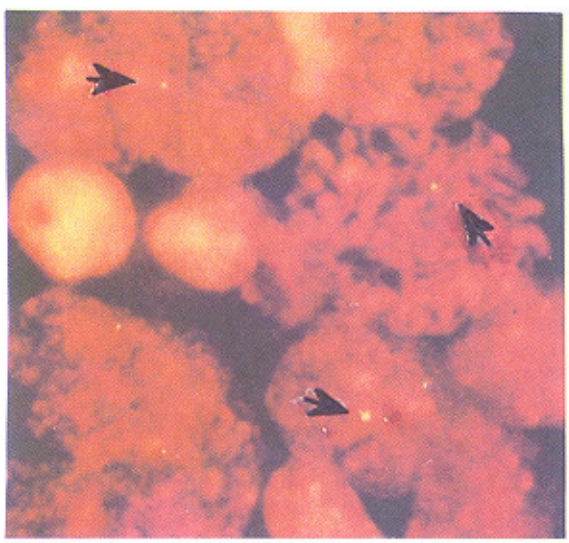

Fig. 1. Breast tumour-interphase nuclei hybridized with DNA-probe for chromosome 3 cen (arrows show the presence of 1 signal individually).

DISCUSSION

In BC, a prefrential loss of chromosome 3p was observed. The distribution of signal count per nucleous was very heterogeneous which indicates a large variability in the tumour karyotype, especailly in ductal carcinomas One of the advantages of FISH is that all cell population of tumours could be analysed. Finally, the concordance of the numerical involvement of chromosome 3 was confirmed. Most frequent signal count revealed to be one spot, either individually (12.7%-58.4%) or totally (33.6%) which followed by 2,3 and 4 signals (Table 1, Fig. 1).

The presence of 2 signals which could be refelctive of diploid mode, was observed in only one of 4 tumours (ID8, 16.6%). The remaining 6 tumours (83.3%) presented 1 spot (Fig. 1) as the most frequent signal-count.

In two tumours (ID 27, ID 33,28.5%), the most frequent signal-count was 3 (33.1%) followed by 4 and more than 6 signals (25.5% and 18.3% respectively) in tumour 27, and 3 signals (43.1%) followed by 1 and 4 signals (30.1% and 17.2% respectively) in tumour 33.

However, the present data could be considered as an initial step for oncogenetic investigation on solid tumours, specifically BC, which only reflects a primary and partial report of our series of patients under genetic investigations in Iran. In conclusion, the application of fluorescence in situ hybridization (FISH) is considered as a powerful detective technique for the chromosome numerical alterations, including chromosome 3 cen in tumour cells of BC patients.

Acknowledgment

The auhtors are greatful to Prof. G. Schwanitz and Dr. B. Roitzheim (Institute of Human Genetics. University. Of Bonn. Germany), for their Scientific advice and technical helps, and also to the Deutsche Akademische Austauch Dients (DAAD) for the financial support.

REFERENCES

1. Marshall E. Search for a killer: Focus shifts from fat to harmones. Science 259: 618-621, 1993.

2. Cremer T, Landegent J, Bruckner A, School HP, Scardin M, Hager HD, Devilee P, Pearson P, Van dv ploeg M. Detection on chromosome alterations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: diagnosis of trisomy 18 with probe L. 1.84. Hum Geret 74: 346-352, 1986.

3. Devilee P, Thiery RF, Kievits T, Killuri R, Hopman AHN, Willard HF, pearson PL, Cornelisse CJ. Detection of chromosome aneuploidy in interphace nuclei from primary breast tumors using chromosome - specific repetitive DNA probes. Cancer Res 48: 5825-5830, 1988.

4. Devilee P, Van Vliet M, Bardoel A, Kievits T, Kuipers Dijkshoorn N, Pearson PL, Cornelise CJ. Frequent somatic imbalance of marker alleles in chromosome in human Primary breast carcinoma. cancer Res 51: 1020-1025, 1991.

5. Cornelisse CJ, Juipers - Dijkshoorn N, Van Vliet M, Hermans J, Devillee P. Fractional allelic imbalance in human breast cancer in cases with tetraploidizalion and chromosome loss. Int J Cancer 50: 544-548, 1992.

6. Dutrillaux B, Gerbautt - Seurean M, Zafarani B. Characterzaation of chromosomal anomalies in human breast cancer. A comparison of 30 Paraadiploid cases with few chromosome changes. cancer Grenet cytogenet 49: 203-217, 1990.

7. Gebhart E, Breuderlein S, Augustus M, Siebert E, Feldner j, Schmidth W. Cytogenetic studies on human breast caarcinomas. Breast cancer Rest treat 8: 125-138, 1986.

8. Teyssier Jr. The chromosomal analysis of human solid tumors. A triple challenge. Cancer Genet Cytogenet. 37: (03-125), 1989.

9. Atri M, Mehdipour P, Mir-Malek A. Family history of neoplasms in 40 paatients with primary breast cancer (in press Med J I.R.I).