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The Definition of nm 23 Protein Expression Phenotype in Breast Carcinoma Cells by Dansitometric - Morphometric - Textural Parameters : An Image Cytometric (ICM) and Consecutive - Double - Staining (CDS) Study by SAMBA 4000

Meme Karsinomu Hücrelerinde nm23 Protein Ekspresyonu Fenotipini Dansitometrik - Morfometrik-Yapısal Parametrelerle Tanımlamak: SAMBA 4000 ile Bir Görüntü Sitometresi ve Ardışık İkili Boyama Çalışması

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Özet: Bu çalışmanın amacı, nm23 protein ekspresyonun fenotipik özelliklerinin nükleus alanı, nükleusun şekil özelliği, DNA içeriği, kromatin heterojenitesi, DNA konsantrasyonu ve kromatin üniformitesi gibi karyometrik parametrelerle tanımlanıp tanımlanamayacağını araştırmaktır. nm23 protein reaktivitesinin saptanması için kullanılan immün sitokimya ve karyometrik parametrelerin ölçülmesi için kullanılan feulgen pararosanilin yöntemi ardışık olarak uygulandı. Değerlendirmeler görüntü sitometresinde yapıldı. Meme kanseri hücrelerinde nükleus alanı, nükleus şekil özelliği ve DNA içeriği ile nm23 reaktivitesi arasında ilişki anlamlı değildi. Kromatin üniformitesi ve DNA konsantrasyonu nm23 pozitif hücrelerde negatif hücrelerdekine göre

Summary: *The aim of this study, was focused on whether the phenotype of nm23 expression can be determined by karyometric parameters including nuclear area, nuclear form factor, DNA content, chromatin heterogeneity, DNA concentration chromatin uniformity and immunocytochemical staining for the detection of nm23 protein reactivity and feulgen pararosaniline techniques for measuring karyometric parameters were consecuted. Analysis was performed by image cytometry. Correlation of nuclear area, nuclear form factor and DNA content with nm23 reactivity in breast carcinoma cells was not significant. Chromatin uniformity and DNA concentration were both significantly lower in nm23 positive cells than they were in nm23 negative cells. In contrast, chromatin heterogeneity was*

önemli derecede düşük iken, kromatin heterojenitesi nm23 pozitif hücrelerde daha yüksekti. Sonuçta, erken evre meme kanserlerinde kromatin üniformitesi, kromatin yoğunluğu ve kromatin heterojenitesi gibi karyometrik parametrelerlerin nm23 protein ekspresyonu (non-metastatik davranış) ile ilişkili olduğu gösterildi.

Anahtar Sözcükler: Meme kanseri, görüntü sitometresi, nm23 protein.

significantly higher in nm23 positive cells than that of nm23 negative cells. In conclusion, karyometric parameters such as chromatin uniformity, chromatin intensity and chromatin heterogeneity, have shown to be associated with nm23 expression (non-metastatic behavior) in early stage breast carcinoma cases.

Key Words: *Breast cancer, image cytometry, nm23 protein*

Although it has been well known that distant metastasis is the main cause of death in breast cancer, the genetic basis of metastasis is not yet well understood. nm 23 protein is an expression of gene which is located in the 17q22 region in human genome and it has been thought to be a metastasis suppression gene (1-3). Many studies have been carried out to find out its chemical-physical nature, gene relation and prognostic importance. Many kind of tumors have been encountered in these investigations as well as breast cancer. While most of them revealed an association between well-differentiated breast cancers, No and Mo conditions and nm23 protein overexpression (4-7), some demonstrated contrary results (8-10).

In this study, our aim was to find out if there is any confidently definable phenotypic karyometric feature for nm23 protein expression.

Materials and Methods

We used imprints of 10 invasive ductal carsinoma - NOS cases (T1 No Mo / AJCC-1994) in this study. All tumors were grade I or II (Scarf-Bloom-Richardson). We stained all cells according to the consecutive double staining (CDS) technique. At the first step of this technique, we stained all cells according to the immunocytochemical procedure for nm23 protein (BioGenex Lab, USA). After evaluating 50 positive stained tumor cells, of their we marked and mapped them on the glass slides and stored their images in the data base. All cytoplasmic positivity regardless of its intensity were taken into account. At the second step of CDS technique, all glass slides which were previously evaluated for nm23 protein expression were treated with Feulgen-pararosaniline staining procedure. Selection of the positive and negative tumor cells for nm23 protein from the imprints of ten tumors was made randomly. We computed nuclear area, DNA content, DNA concentration (MOD = IOD / NA), chromatin heterogeneity (VOD = sqrt (IOD - MOD) / NA), chromatin uniformity and nuclear form factor parameters, by using SAMBA 4000 Image Cytometry (ICM) software, in both previously marked nm23 positive cells and randomly selected 200 nm23 negative cells.

NFF is the compactness of nucleus which is often known as $(Perimeter)^2 / 4\pi$ Area. A perfectly circular nucleus has a NFF value of 1; as the shape of the nucleus departs from circularity, NFF increases. By means of densitometric analysis, the ICM software computes the optical density (OD) for Feulgen stain for each pixel within the entire nucleus and calculate them. The result of this calculation is known as integrated optical density (IOD) and reflects DNA content of target nucleus. ICM defines the optical density (OD) of each pixels within the entire nucleus and that known as GLD and shows chromatin uniformity.

Non-parametrical test of ANOVA were performed to compare the findings of both nm23 positive and negative group of cells.

Results

All patients were at postmenoposal period with an age ranging from 54 to 62 (average: 61.2). Findings of nm23 positive (Figure 1) and nm23 negative cells are shown in Table I. Although there was no significant difference between nm23 positive and negative cells with respect to NA, NFF, and DNA content ($p>0.05$), these two group of cells were significantly different by means of chromatin uniformity, DNA concentration, and chromatin heterogeneity ($p<0.05$), (Figure 2).

Table I. Findings of nm23 positive and negative cells.

		nm23 positive cells (n=50)	nm23 negative cells (n=200)
NA (µm2) (Nuclear Area)	min	284.71	254.27
	max	527.42	498.59
	A±SD	376.24 ± 157.17	381.05 ± 149.48
NFF (Nuclear Form Factor)	min	1.835	1.529
	max	2.612	2.130
	A±SD	2.197 ± 0.526	2.411 ± 0.603
IOD (DNA content)	min	24403	32291
	max	48090	81838
	A±SD	39559.21 ± 6585.62	48065.93 ± 13115.2
GLD (Chromatin uniformity)	min	9.72	9.18
	max	13.46	21.07
	A±SD	10.94 ± 2.87	15.13 ± 6.97
VOD (Chromatin heterogeneity)	min	0.11	0.19
	max	1.91	1.26
	A±SD	0.91 ± 0.09	0.51 ± 0.04
MOD (DNA concentration)	min	11.75	19.41
	max	24.11	31.48
	A±SD	13.41 ± 4.71	23.14 ± 9.18

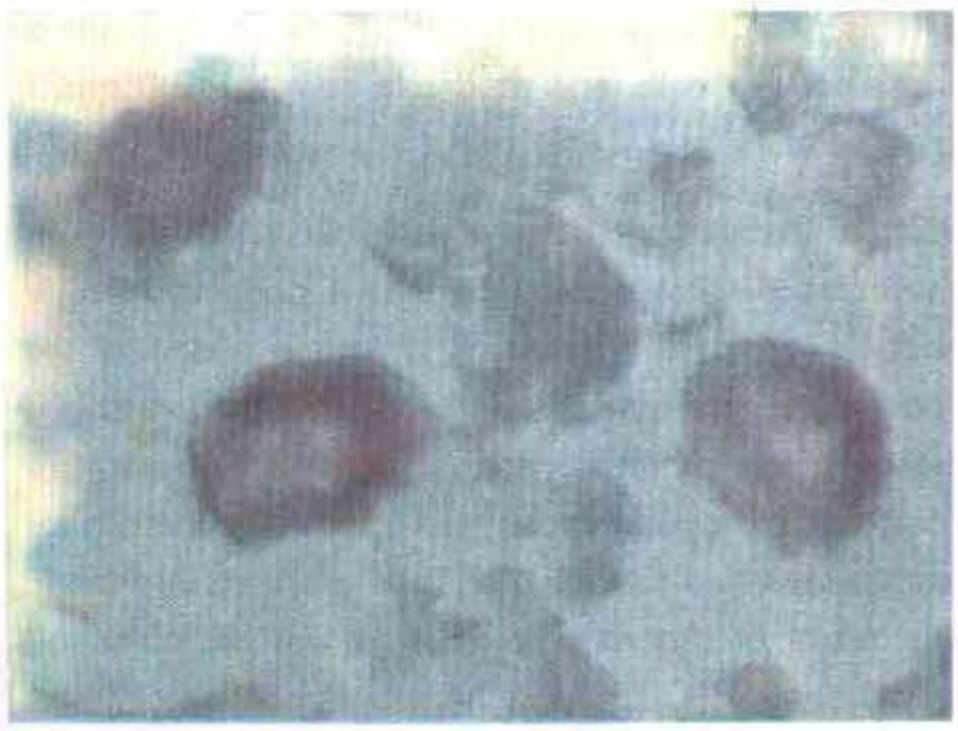

Figure 1. nm23 positivity in tumor cell cytoplasm (x20 objective, Digital image, IPS/ SAMBA).

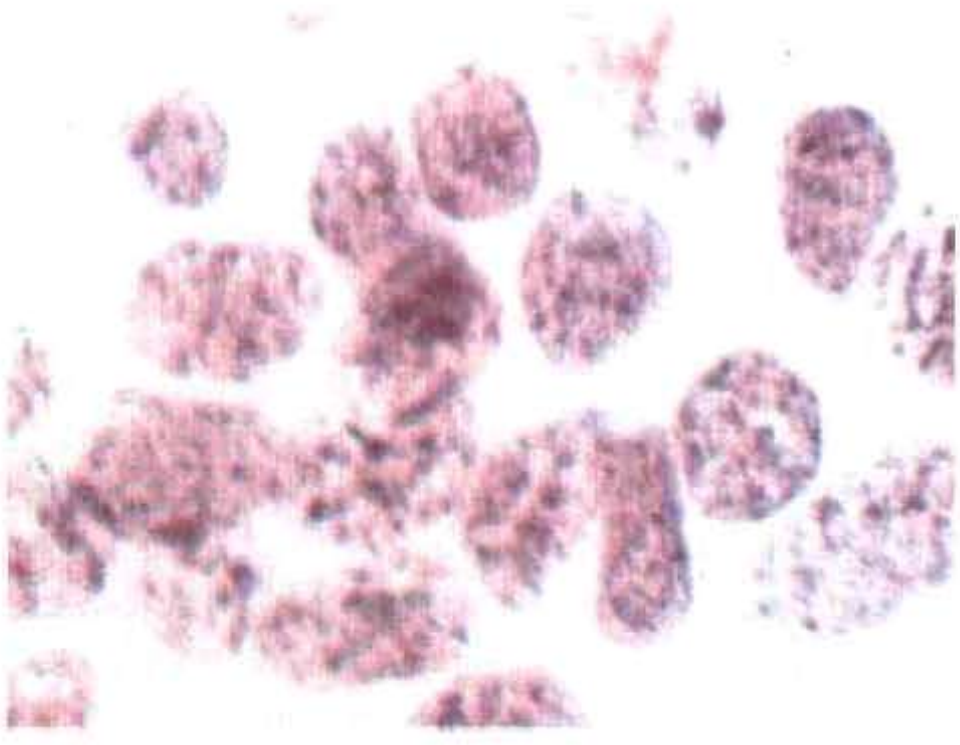

Figure 2. Feulgen-Pararosaniline stained breast cancer cells (x20 objective, Digital image, IPS/ SAMBA).

Although, DNA content and nuclear area were not found significant for nm23 protein expression phenotype, their ratio defines the chromatin concentration (= Mean Optical Density = DNA content / NA) which have an importance for identifying nm23 positive cell.

In our study, IOD values were not found significant for the definition of nm23 protein expression phenotype ($p>0.05$). However, OD values of each pixel within a nucleus were found significantly more uniform in nm23 positive cells than that of nm23 negative cells ($p<0.05$).

The distribution of OD values were significantly heterogenious in nm23 positive cell nucleus ($p<0.05$). This variance of OD [(IOD-MOD) / NA] values reflects the heterogeneity of chromatin.

Discussion

Since the human being has a unique genome, each cell type differentiates on the basis of its own genotypic feature. This genomic character reflects somewhat different or unique phenotype which is mostly definable by today's conventional / advanced technology. However, there are some cells which have different genotypic features but same phenotype. Since the nm23 protein expression is one of the genotypic characteristics of cell lineage, it should possibly have a phenotypic feature. To clarify this hypothesis, we investigated whether nm23 gene overexpression has a different or unique karyometric feature. For this purpose, we created a model that provides the measurement of karyometric features including the nm23 protein expression in the same target nuclei. The consecutive double staining procedure seemed to be the best method for this purpose. Although, there are many studies on nm23 protein, dealing with its chemical-physical nature, gene relation and prognostic importance(1-8), to the best of our knowledge, there is no study aimed to define phenotypic karyometric features associated with this protein expression in English literature.

In our study a significant correlation was not found between nm23 expression and NA, NFF and DNA

content in breast carcinoma cells. However, chromatin heterogeneity, DNA concentration and chromatin uniformity was significantly different in cells with positive and negative nm23 protein reactivity. When the nuclei of cells expressing nm23 protein are examined morphometricly, their chromatin was found uniform in density, heterogenous in distribution and low in concentration. In contrast, nm23 negative cells showed no chromatin uniformity, no chromatin heterogeneity as well as high chromatin concentration. These karyometric findings may be distinguished features which reflect different biologic nature. Most probably these features may not be reflected in conventional light microscopic preparations such as PAP or H + E stained slides.

In conclusion, karyometric parameters such as MOD, VOD and GLD, measured by ICM, are of important value to identify nm23 expression (non-metastatic behavior) in the early stage of breast carcinoma. They may not also be expressed by morphometric parameters such as NA, NFF and DNA content. Further investigations by combining immunocytochemical panels of well-known prognosticators and image cytometric parameters seem to have an important roles in the evaluation of metastatic behavior, especially in No cases which are still under discussion for giving adjuvant chemotherapy.

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