

**UNIVERSITY OF MEDICINE AND PHARMACY  
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# **Phd THESIS**

***THE STUDY OF MODIFICATIONS  
OF THE TUMOR MORPHOLOGICAL  
PROFILE IN RELATION TO THE  
DEGREE OF DIFFERENTIATION IN  
PROSTATIC CARCINOMA***

## **ABSTRACT**

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## KEY WORDS

Prostate adenocarcinoma, Fractal Dimension, Biological behavior, Gleason system, Srigley system.

## CURRENT STATE OF KNOWLEDGE

### CLINICO-EPIDEMIOLOGICAL PROFILE

Prostate carcinoma (PC) is a malignant epithelial proliferation that develops from the epithelium of prostatic acini and/or ducts and presents extremely variable glandular differentiation, anaplasia, and biological behavior.

Today, PC is, according to official WHO data until 2012, the second most frequent and most diagnosed neoplasia in men, surpassed only by lung cancer and the fourth most frequent neoplasia in general, being surpassed by lung, breast and colorectal cancers [Stewart and Wild 2014].

Mortality and incidence of prostatic epithelial malignant neoplasia varies, however, significantly from one geographic region to another. Survival rate of PC is much smaller in developing countries than in developed countries due to late diagnosis and lack of access to adequate healthcare systems [Jemal et al 2011]. The Incidence and Mortality of PC not only present geographical differences but also time variations. Wang and his colleagues identified six types of profiles for the evolution of incidence and mortality by PC [Wong et al 2016].

In our country, the incidence of PC among cancers in men is in third position, below lung cancer and colo-rectal cancer, while in Europe it occupies the first position [Ferlay et al 2013].

Although the etiology of CP is not yet fully elucidated, numerous epidemiological studies having outlined, over time, an extremely wide and varied range of factors that have been considered to be associated with the risk of developing CP. They can be grouped into individual, genetic and behavioral and environmental factors. Of these, however, only very few, namely age, family history and race, have been shown to have a direct relationship with the occurrence of cancer [Haas et al 2008; Sierra et al 2016].

A more didactic classification of the factors related to the risk of developing CP divides them into two categories [Sadeghi-Gandomani et al 2017] namely:

- Major risk factors
- Factors with less clear effect on the risk of developing CP (minor risk factors)

Clinical manifestations are detectable only after the age of 60-70 years [Abate-Shen and Shen 2000]. It appears that some PCs may undergo a latency period of up to 15-20 years, during which time the disease is present histologically but does not cause clinical manifestations [Haas et al 2008]. With all the variety of symptoms, in almost half of the cases, the patients with prostate cancer are asymptomatic.

To establish a correct and rapid diagnosis, the following protocol is currently used:

- For tumor diagnosis [NCCC 2014]: Digital Rectal Examination (DRE), Prostate Specific Antigen (PSA) value, Transrectal ultrasound (TRUS) and Biopsy followed by histopathological examination. The subsequent progress of the algorithm depends on the clinical staging established after the tumor diagnosis stage is completed.
- For stage diagnosis: Imaging evaluation: (Lung radiography, Urography), Bone scintigraphy, Abdominal ultrasound and Biochemical, biological investigations, Tumor markers

It should be noted, however, that excessive PC discovery also has obvious adverse clinical consequences, since most patients do not experience direct clinical benefit from treatment [Hoffman et al. 2014]. It has been estimated that between 42%

and 66% of diagnosed cancers would not have been clinically affected if they had not been detected [Draisma et al 2009].

Predictive factors of the prognosis can be divided into two categories [Hayes et al 1996]:

- **Predictive factors** – predict the response or resistance to a specific treatment
- **Prognostic factors** – predict the relapse or progression independent of the future effects of the treatment and they are divided, in turn, into three categories categories: **Category I** – Established factors, recommended for routine reporting; **Category II** – Factors that promise or are recommended, despite incomplete data; **Category III** – Factors that, at present, are not recommended because of insufficient data

Several systems for evaluating the evolution and prognosis of PC have been proposed. In all of these, the tumor-related factors used were the same three of the category I described above, namely: serum PSA level at diagnosis, clinical staging (TNM), histopathological staging (Gleason Score) to which the positivity of the tumor margins was added. [Martin et al 2011, Stephenson et al 2009, D'Amico et al 1998, Kattan et al 1999].

Although, unlike other solid tumors, PC is not characterized by frequent mutations that have predictive or prognostic value, many molecular and genetic factors have been explored to attempt a more accurate individualization of risk prediction [Martin et al 2011, Kan et al 2010].

The most used system in medical practice was the TNM system which uses several descriptive parameters [Catalona 1984; Fleming et al 1997]. In the 8th edition of the TNM Classification of malignancies [Brierley et al 2017], the TNM system of clinical staging was supplemented and combined with the two prognostic factors validated by the practical clinical experience, namely the PSA value and the evaluation by the Gleason system.

## **MORPHOLOGICAL PROFILE**

Prostate is an epithelial "budding" that occurs in the intermediate (pelvic) region of the primitive urogenital sinus (whose epithelium is derived from the endoderm), immediately below the bladder [Abate-Shen and Shen 2000; Cunha et al 1986].

Embryogenesis presents two phases in all vertebrates: Phase I, called ambisexual or Indifferent phase; Phase II or Phase of Sexual Differentiation

Genetic studies on the Nkx3.1 homeobox gene have shown that regions of the primitive urogenital sinus have the ability to differentiate in order to form the prostate [Bhatia Gaur et al. 1999]. Several candidate genes have been identified for the role of regulating prostate development.

At present, it is accepted to divide the prostate into four fundamental anatomical regions or compartments [McNeal 1981; Aumuller 1989 a and b], the central anatomical reference point being represented by the relationship between each of these regions and the urethra: Peripheral area; Central area; Transition area or preprostatic segment; Anterior non-glandular fibromuscular stroma.

The adult prostate is made up of two fundamental components: the parenchyma consisting of 30-50 individualized glands and a non-glandular component that constitutes the stroma of the organ.

The epithelial compartment that forms the glandular structures of the prostate is made up of at least three different cell types that can be distinguished due to their morphological features, functional significance and relevance to carcinogenesis. These are: exocrine secretory cells, basal and neuroendocrine cells to which stem

cells, and transitional-type epithelial cells were added [Abate-Shen and Shen 2000; Stamey and McNeal 1992; McNeal 1976].

The stromal compartment is a complex structure that forms the environment for mechanical and trophic support of functional epithelial cells. It is composed, in turn, of two basic components: the specific cellular component, dominated by the smooth muscle fibers and the extracellular matrix [Aumuller 1983; Mawhinney 1989; Hayward 2002; Brar et al 2003; Saito and Munakata 2004; Laczko et al 2005; Long et al 2005].

At present, the major histopathological features of prostate adenocarcinoma are recognized and accepted by most pathologists especially when it comes to specimens obtained by needle biopsy. From the entire range of the assessed histopathological features, the nuclear modifications - the large dimensions of the nucleus and the nucleoli highlighting - and the architectural disposition pattern with infiltration character equal to or greater than "3" are the ones that stand out [Kumar et al 2015].

In 1966, Donald Gleason developed the prostate adenocarcinoma evaluation system that defines five histological patterns (stages) of differentiation. The Gleason 1 pattern represents the stage with the best differentiated appearance and is correlated with the most favorable prognosis, while the Gleason 5 pattern is the stage with the least differentiated appearance and is associated with a very poor prognosis [Gleason 1966]. Gleason detailed and summarized the models (degrees) of histological differentiation of prostate adenocarcinoma, analyzing their correlation with clinical data such as staging and prognosis [Delahunt et al 2012; Chen and Zhou 2016].

Towards the end of 2014, an international meeting was held in Chicago with the aim of updating the Gleason system, a meeting attended by not only pathology experts but also urologists, oncologists and radiotherapists [Pierorazio et al 2013; Epstein et al 2016a; Kryvenko și Epstein 2016].

In 2004, John Srigley, starting from the reality of the great heterogeneity of the features of PC that can easily create confusion at the time of diagnosis with many other pathological processes, also proposes a simpler system of dividing the tumor architectural aspects that groups the wide range of patterns described by Gleason. The author also reviews the normal morphological aspects or non-neoplastic pathological processes that may create differential diagnosis problems with malignant prostatic epithelial proliferation [Srigley 2004].

Over time, there have been numerous attempts to define and classify histological variants of CP. One of these approaches [Humphrey 2012] divides CP into two groups: acinar adenocarcinoma and its histological variants and types of non-acinar adenocarcinoma. The vast majority of prostate cancers are acinar adenocarcinomas. It is important, however, that pathologists also recognize rare histological variants of acinar adenocarcinoma [Humphrey 2012; Li and Wang 2016].

The appearance and progression of the malignant cellular phenotype in the population of epithelial cells of acini and prostate ducts is the result of a dynamic process of dramatic genetic changes of normal epithelial cells and then of tumor cells consisting of successive chromosomal alterations that release them from regulatory signals that limit their ability to proliferate and invade nearby structures, probably by reducing or losing the function of the genes that are supposed to be suppressors of the tumor process [Abate-Shen and Shen 2000; Dvorak 2015]. This complex process of gene alteration comprises several stages, namely: the Initiation Stage, the Progression Stage, and the Advanced Progression Stage with the onset of metastases.

Tumor stroma from cancers, also known as reactive stroma, has many features similar to stroma in wound healing areas, elements that have been identified in breast or colon tumors or even prostate carcinoma [Bhowmick and Moses 2005; Dvorak 1986; Rowley 1998-1999; Tuxhorn et al 2001].

Morphological changes at the epithelial level, consisting of the loss of normal tissue architecture, nuclear atypia and genetic changes [Niu and Xia 2009] are accompanied by the following fundamental morphological changes of the reactive stroma of prostate malignant tissue: significant decrease or disappearance of smooth muscle cells as the cancer progresses, miofibroblast and fibroblast proliferation, proper ECM amplification, angiogenesis amplification and genetic alterations.

Image analysis represents the extraction of useful information from microscopic images, usually acquired using a video camera or a histological slide scanner. The applications of image analysis in pathology, both in the research area and in the diagnostic area are extremely numerous and varied.

A fractal is an object/geometric form made up of subunits that represent small copies of the original structure and which have a behavior similar to the original one when examined at different scales. This feature is called self-similarity [Chaudhuri and Sarkar 1995; Waliszewski et al 2010]. Fractal dimension analysis has begun to be used in many medical specialties such as cardiology, neurology, ophthalmology and radiology because computer-based image analysis allows a truly quantitative approach and is hopefully reproducible, becoming a useful tool for experienced researchers [Keipes et al 1993; Baish and Jain 2000]. In male urogenital pathology, fractal dimension analysis was used both in the study of prostate tumor tissue [Tambasco et al 2009; de Arruda et al 2013; Waliszewski et al 2010; 2014; 2014b; 2015; 2016a; 2016b; Tanase and Waliszewski 2015; Stepan et al 2015] and of the vascular network in prostate parenchyma and prostate tumors [Taverna et al 2009, 2015].

## **PERSONAL CONTRIBUTION**

### **MATERIALS AND METHODS**

The study base of the present work consisted of a group of 109 patients hospitalized with the diagnosis of prostate carcinoma who underwent radical prostatectomy.

The study material consisted of tissue fragments of prostate parenchyma obtained from radical prostatectomy specimens and included in paraffin.

The study was prospective in that it only included patients diagnosed with prostate carcinoma found on the total prostatectomy pieces after its start and included two major research directions:

I. Assessment of tumor architecture using fractal dimension (FD)

II. Assessment of biological behavior (BB) of malignant tumor cell populations.

Two different study groups were created for the two proposed research directions consisting of 453 and 435 tumor fields respectively with distinct architectural patterns. The features of tumor architecture were evaluated separately and subdivided into subgroups using two systems for staging the degree of tumor differentiation, namely: the Gleason system and the Srigley system.

For each of the two research directions, a set of three parameters were considered, as follows I. For **tumor architecture**: (1) Tumor cell population architecture; (2) The architecture of the stromal support structure; (3) The

architecture of the vascular network. II. For **biological behavior**: (1) Degree of adhesion between malignant cells; (2) The degradation capacity of the extracellular matrix; (3) The degree of aggression.

The human biological material represented by the prostate tissue fragments was subjected to classical histological processing techniques. From the obtained paraffin blocks four sections were made with a thickness of 3-4  $\mu\text{m}$ . The first section was stained using Hematoxylin - Eosin (H-E) after which, between 1 and 5 fields of interest were selected, fields that presented compact tumor proliferation, without necrosis, containing a distinct architectural pattern from Gleason's classification modified by us [Gleason 1977] and then converted also to Srigley's system [Srigley 2004]. The fields of interest were delimited on the slide using a marker pen.

The primary paraffin blocks were punctured using a steel cylinder device, sharpened on the inside, 2.5 mm in diameter, at the areas of interest identified and marked on the Hematoxylin - Eosin colored slides. The obtained paraffin cylinders were mounted in "acceptor" blocks made of a patented composite material that simulates the physico-chemical properties of the animal tissues and then embedded in paraffin using the usual methods [Muşat 2017]. The blocks thus obtained were sectioned at a thickness of 5  $\mu\text{m}$ , with 4 serial sections selected from each block.

The three serial sections obtained from the primary blocks outside the first Hematoxylin - Eosin stained section were stained in each case according to the algorithm in Table 1.

**Table 1: Staining algorithm used for the assessment of tumor architecture**

Section	Staining	Aim
S 1	<i>H-E</i>	Establishing Gleason/Srigley patterns
S 2	<i>Silver impregnation (Gömöri method)</i>	Assessment of tumor architecture (GÖ)
S 3	<i>Tricromic Goldner</i>	Assessment of stromal architecture (TC)
S 4	<i>CD34 immuno-staining</i>	Assessment of vascular architecture (VN)

The 4 serial sections obtained from the secondary microarray blocks were immunohistochemically labeled according to the algorithm in Table 2.

**Table 2: Immuno-staining algorithm used for the assessment of biological behavior**

Section	Staining	Aim
S 5	<i>MMP9</i>	Evaluation of the degradation capacity of the extracellular matrix
S 6	<i>MMP2</i>	
S 7	<i>ECAD</i>	Assessment of intercellular adhesion
S 8	<i>PTEN</i>	Molecular evaluation of aggression/prognosis

All histological specimens obtained both by classical staining and staining with immunohistochemical markers were transformed into virtual slides with a Leica Aperio AT2 scanner, using: (a) the X20 objective for the 4 serial sections used for the evaluation of tumor architecture and (b) and the X40 objective for the 4 sections series used to evaluate the biological behavior of prostate epithelial malignancies.

**Evaluation of tumor architecture using FD.** Starting from the H-E colored guidance slide, each field of interest was identified on the other three serial stainings. From each field of interest a square field containing the same area of tumor tissue with dimensions of 512/512 pixels to optimize the FD calculation algorithm was extracted. FD determination for the three components of the tumor architecture was

performed using algorithms that were designed in the MATLAB program (MathWorks, USA).

The process of computerized image evaluation went through two stages:

- I. Processing of the images which consisted of two steps: (a) Segmentation and (b) Binarization and
- II. Calculation of fractal dimension (FD) of binary images.

Two BOX-Counting algorithms were tested for FD estimation. Reporting was done using Algorithm I.

**Assessment of biological behavior.** From the immunolabeled virtual slides, RGB images from the same field with homogeneous pattern of tumor architecture were selected, each image having the same area of 1778582 pixels.

The algorithm for assessing the intensity of immunostaining with the four antibodies was applied in two steps: I. Selection of the regions of interest (ROI) and II. Computational assessment of each characteristic of biological behavior.

The image analysis included several stages: ROI was defined within the original image as the set of pixels that simultaneously complied with the following two rules:

- (1) The value of the "Green" channel was not higher than the fixed threshold of 220;
- (2) The value of the "Red" channel was at least 1.1 times higher than that of the "Blue" channel. All other pixels were marked "0" on all channels.

The mean value of the "Grayscale" version of ROI was considered the value of determining the immunohistochemical staining. Because the acquisition technique was based on transmitted light, each determined value was subtracted from "255" so that the value of the determination increases with the staining intensity.

The scale of immunostaining intensities was set between "0" (colorless) and "90" (maximum threshold, lowered from "255" to "90" because there were no values higher than the threshold of "90" and to optimize the graphic expression of the results).

For data collection, "database" files were created in the computer. Preliminary data processing was performed using the Microsoft Excel module from the Microsoft Office 2010 Professional software package. The statistical indicators calculated for the numerical parameters were: The lowest value (VMIN); Highest value (VMAX); Average value (AV); Standard deviation (STDEV). In order to be able to perform the statistical analysis of the numerical values, it was first necessary to evaluate the normality of the numerical data ranges of each parameter using the Lilliefors test.

The statistical evaluation had two directions:

(I) Comparison of the difference between the AV of the studied parameters that used the following tests:

- 1, For Normal distributed series of values: the "t" test (Two-Sample Assuming Equal Variances) and the One-Way ANOVA - Single Factor Test
- 2. For Not Normal distributed series of values: the Mann-Whitney / Two-tailed test and the Kruskal-Wallis Test;

(II) Measurement of the intensity and direction of the association between the values of two of the studied parameters that used the "Pearson's" correlation test.

Diagrams (graphs) illustrating evolution trends of different assessed parameters, as well as statistical comparisons between them have been done using the "Graph" tool from "Word" and "Excel" modules of the Microsoft Office 2010, 2013 and 2016 Professional software packages as well as the XLSTAT 2014 "add on" program for the "Excel" module, trial version.

## **STUDY OF TUMOR ARCHITECTURE USING FRACTAL DIMENSION**

In the **Gleason system**, the AV of FD of the **tumor cell architecture** showed a stabilizing tendency, with a discrete downward slope from well-differentiated to poorly differentiated patterns, against a background of oscillation of these values from one subtype to the other, apparently without any a kind of connection with the degree of differentiation.

The AV of FD of the **stromal architecture** showed an obvious upward trend from the well differentiated to the poorly differentiated patterns, against a background of more attenuated oscillation of these values from one subtype to the other.

The AV of FD of **the vascular network architecture** as a whole showed an obvious downward trend from the well differentiated patterns towards the poorly differentiated ones, although they started from low values for the well differentiated patterns, ascending to the moderately differentiated patterns and then returning to low values for the poorly differentiated patterns.

In the **Srigley system**, probably due to the grouping of the subtypes in the Gleason system, the AVs of the FD of the three components of the tumor architecture generally showed clearer trends of evolution from the well differentiated to the poorly differentiated patterns with a notable exception, namely to the architecture of the tumor cell population in which the trend was obviously an ascending one, compared to the discrete descending one, observed in the case of the Gleason System.

Comparison of the AVs of FD of the three components of the tumor architecture, both at the level of the whole group and separately on the value ranges of each subtype of the Gleason and Srigley systems, revealed significant differences, statistically validated (very significant "p") between the AVs of the three components. in both systems of assessment of the degree of differentiation.

The correlation diagrams between the AVs of the FD of the three components of the tumor architecture for the whole group revealed that the AVs of the FD of the **GÖ** and **TC** for the whole group expressed a net tendency of statistically validated direct correlation (the calculated value of "p" - very significant). In other words, the architectural arrangements of GÖ and TC evolve simultaneously towards either the area type or the linear type.

The AVs of FD of **GÖ** and **VN** for the whole lot expressed a net trend of statistically validated direct correlation (calculated value of "p" - highly significant). In other words, the architectural arrangements of GÖ and VN evolve simultaneously towards either the area type or the linear type.

The AVs of FD of **TC** and **VN** for the whole lot expressed a vague tendency of direct correlation (calculated value of "p"> 0.05 but <0.5). In other words, the architectural arrangements of TC and VN tend to evolve simultaneously towards either the area type or the linear type.

The correlation diagram of the three components of the tumor architecture allows to define architectural profiles for the four types of assessed patterns of the Gleason system. Thus:

- The Gleason 2 pattern shows a discrete tendency of area disposition model for tumor cell architecture, shows a more pronounced tendency of disposition of the tumor stromal architecture on a linear model and a more pronounced tendency of disposition of the intratumoral vascular architecture on a linear model
- The Gleason 3 pattern shows a more pronounced pattern of area disposition model for tumor cell architecture, presents a tendency of intermediate

disposition for the tumor stromal architecture and a tendency of disposition on a linear pattern for the intratumoral vascular architecture

- The Gleason 4 pattern shows a tendency of area disposition model for the tumor cell architecture, presents a discrete tendency of linear model orientation for the disposition of the tumor stromal architecture and an obvious tendency of disposition of the intratumoral vascular architecture on a linear model

- The Gleason 5 pattern shows an obvious tendency of disposition on an area model for tumor cell architecture, a discrete tendency of disposition of the tumor stromal architecture on an area model and the most pronounced tendency of disposition of the intratumoral vascular architecture on a linear model.

For the four types of patterns of the Srigley system, the sketches of architectural profiles would be as follows:

- The Srigley 1 pattern shows the most pronounced tendency towards an area disposition model for tumor cell architecture, right below Srigley 4 pattern, it shows a discrete tendency of disposition of tumor stromal architecture on a linear model and a moderate tendency of disposition of the intratumoral vascular architecture on a linear model.

- The Srigley 2 pattern shows a clear tendency of disposition on an area model for tumor cell architecture, it shows a clear tendency of intermediate disposition of the tumor stromal architecture and a moderate tendency of disposition on a linear model of the intratumoral vascular architecture.

- The Srigley 3 pattern shows a clear tendency of disposition on an area model for tumor cell architecture, it shows a clear intermediate disposition tendency with a vague orientation of the disposition of the stromal tumor architecture on a linear model and the least pronounced tendency of disposition of the intratumoral vascular architecture on a linear model of all patterns

- The Srigley 4 pattern shows, as we have indicated, a special position, with the highest tendency of disposition on an area model for the tumor cell architecture, the highest tendency of disposition on an area model of the tumor stromal architecture between all of the patterns and the highest tendency of disposition of the intratumoral vascular architecture on a linear model.

## **STUDY OF THE BIOLOGICAL BEHAVIOR OF TUMOR CELL POPULATIONS**

In the Gleason system, the AV of the intensity of **MMP9 expression**, as an indicator of the degradation capacity of the extracellular matrix by the tumor cells, showed a stabilizing tendency, with a very discreet upward slope from the well differentiated to the poorly differentiated patterns, against a background of oscillation of these values from one subtype to the other, apparently without any connection with the degree of differentiation.

The AV of the intensity of **MMP2 expression** also showed a level of stabilization around a threshold almost twice lower than the level of MMP9 expression, without any tendency of evolution from the well differentiated to the poorly differentiated patterns and with oscillations from one subtype to the other. more attenuated than in the case of MMP9.

The AV of the intensity of **ECAD expression** as a whole showed an obvious downward trend from the well differentiated to the poorly differentiated patterns, oscillations generally attenuated from one subtype to the other.

Finally, the AV intensity of **PTEN expression**, as an indicator of tumor aggression, also showed, as the AV of MMP9, a stabilizing tendency, with a discrete upward slope from well differentiated to poorly differentiated patterns, against a background of significant oscillation of these values from one subtype to the other, without any relation to the degree of differentiation.

In the Srigley system, probably due to the grouping of the subtypes of the Gleason system, the AVs of the intensity of expression of the four descriptive parameters of the biological behavior of the malignant cell populations generally showed the same evolutionary trends as in the Gleason system but more clearly outlined, stabilizing, without any trend of evolution from well differentiated to poorly differentiated patterns.

Here there was also a notable exception, namely the intensity of ECAD expression, in which the trend was obviously a downward one, as in the Gleason system, from the well differentiated to the poorly differentiated patterns.

Comparison between the AVs of the expression of the immunostaining intensity for each of the four descriptive parameters of the biological behavior, both at the level of the whole lot and separately on the value ranges of each subtype of the Gleason system, revealed significant differences between the AV of the four parameters, differences that were statistically validated ("p" very highly significant).

Also, there were notable statistically validated (very highly significant "p") differences, when comparing AV of the expression of immunostaining intensity for each of the four descriptive parameters of biological behavior taken both two for the whole lot as well as separately by value strings of each subtype of the Gleason system for: MMP9 and MMP2, MMP9 and ECAD, ECAD and PTEN, MMP2 and ECAD and MMP2 and PTEN. In the case of AV comparison of the MMP9 and PTEN expression, significant statistically validated differences were observed, with the "p" value calculated for the Pearson test being "very highly significant" only for the whole lot and for the GL3A, GL3C, GL4CR and GL4FU patterns.

In the Srigley system, the AV comparison of the expression of the immunostaining intensity for each of the four descriptive parameters of the biological behavior between them both for the whole lot as well as separately by the value strings of each pattern also revealed notable, statistically validated ("p" very significant), differences, between the AVs of the four parameters.

Also, there were notable statistically validated (very highly significant "p") differences, when comparing the AV of the expression of immunostaining intensity for each of the four descriptive parameters of biological behavior taken both two for the whole lot and separately on the value strings of each pattern for: MMP9 and MMP2, MMP9 and ECAD, ECAD and PTEN, MMP2 and ECAD and MMP2 and PTEN. Also, in the Srigley system, in the case of AV comparison of the expression of MMP9 and PTEN, significant statistically validated differences were observed, with the "p" value calculated for the Pearson test being "very highly significant" only for the whole lot and for the SG2 and SG3 patterns.

The diagrams of the correlations between AVs of the four descriptive parameters of the biological behavior (MMP9, MMP2, ECAD and PTEN) taken two for the whole lot revealed the following aspects:

- The expressions of both matrix metalloproteinases showed a strong direct correlation with the ECAD expression, both correlations being statistically validated (the calculated value of "p" - very highly significant and highly significant respectively). In other words, the expression intensity of both matrix metalloproteinases evolves in the same sense as the intensity of the expression of the cell adhesion, either towards increase or towards reduction.

- Also a strong direct correlation was shown by the expression of ECAD and the expression of PTEN, correlation that was statistically validated (the calculated value of "p" - significant). In other words, the intensity of cell adhesion expression and the degree of tumor aggressiveness evolve in the same sense, either increasing or decreasing together.
- Also, the MMP9 expression showed a strong, direct and statistically validated (the calculated value of "p" - highly significant) correlation with the PTEN expression. In other words, the intensity of matrix metalloproteinase expression evolves in the opposite direction with the degree of tumor aggressiveness, when the first is increased, the second is reduced.
- Finally, the expression of PTEN showed a statistically validated direct correlation (calculated value of "p" - significant) with the value of the MMP9/MMP2 ratio. In other words, tumor aggressiveness evolves in the opposite direction with the value of the ratio of the intensities of the two matrix metalloproteinases (because the intensity of immunostaining for PTEN evolves in the same sense as the value of the ratio).
- Expression of the two matrix metalloproteinases showed a tendency for direct correlation (calculated value of "p" > 0.05 but < 0.5). In other words, the expression intensities of both matrix metalloproteinases tend to a parallel evolution in the same sense, either towards increase or reduction.
- Also, the ECAD expression showed a trend in direct correlation (calculated value of "p" > 0.05 but < 0.5) with the value of the MMP9/MMP2 ratio. In other words, the intensity of cell adhesion expression tends to a parallel evolution in the same sense with the value of the ratio between the intensities of the two matrix metalloproteinases, either towards increase or reduction.
- Finally, the expression of MMP2 showed only a direct correlation pattern with the intensity of the PTEN expression (calculated value of "p" > 0.5). In other words, the intensity of matrix metalloproteinase expression has a discrete trend of divergent evolution with tumor aggressiveness.

The evaluation of the descriptive parameters of the biological behavior of the epithelial malignant neoplastic proliferation of the prostatic parenchyma revealed some interesting aspects:

The expression of the degradation capacity of the extracellular matrix was dominated by MMP9 - Gelatinase B. The intensity of the expression of MMP9 had an oscillating tendency but with a general discrete upward trend from the well differentiated patterns (Gleason 2 in the Gleason system and Srigley 1 in the Srigley system) to the moderately and poorly differentiated patterns (Gleason 3 and 4, respectively Srigley 2 and 3).

Interestingly, the expression of MMP9 was reduced to the weakest differentiated patterns (Gleason 5, respectively Srigley 4), but the average values of the intensity of the expression were above the average values recorded in the well differentiated patterns.

Evaluation of MMP2 expression showed that Gelatinase A plays a secondary role in extracellular matrix degradation in prostate carcinoma evolution, with MMP9 / MMP2 ratio values ranging from 1.8 to 2.

The expression of ECAD, one of the main actors contributing to the intercellular adhesion, showed, although it is against the backdrop of an oscillating evolution, an obvious downward trend in the intensity values from the well differentiated patterns (Gleason 2 in the Gleason system and Srigley 1 in the Srigley system) to the weakest differentiated patterns (Gleason 5, respectively Srigley 4), indicating a decrease in intercellular cohesion as the degree of differentiation is reduced.

Finally, the expression PTEN, also characterized by significant oscillations from one pattern to another, did not seem to sketch any correlation with the degree of differentiation, although in the patterns of the Gleason system it outlined a slightly upward trend which, however, flattened out when assessing the mean values of the expression in the patterns of the Srigley system.

In general, the trends of the evolution of the analyzed parameters were more clearly outlined in the patterns of the system designed by Srigley.

## CONCLUSIONS

Our study, comprising both individual and comparative analysis of complex tumor architecture and biological behavior of tumor cell populations, led to some conclusions that may be of importance and applicability in medical practice:

The **components of the tumor architecture** presented, as a whole, two categories of trends in the evolution of the arrangement mode expressed by the fractal dimensions (FD) of each of them within the tumor remodeling process: on one hand the evolution trends correlated with the degree of tumor differentiation and on the other hand, the tendencies of mutual influence.

- Regarding the correlation with the degree of differentiation, some interesting aspects have stood out:
  - The disposition of the tumor cells as a whole showed, although on an oscillating background, a tendency to stabilize more around an area type of arrangement of the tumor cell population, with a tendency to evolve towards the pattern of area disposition as the degree of differentiation expressed in the Srigley system decreases.
  - The stromal arrangement also had the same tendency of orientation towards the area type, but lower than in the population of tumor cells, but with a tendency expressed by evolution towards the area type of arrangement, as the degree of differentiation decreases both in the Gleason system as well in the Srigley system.
  - The vascular network generally showed a tendency of arrangement on a linear model, the mean FD values for each pattern in both classifications being less than 1.4. Moreover, this tendency has increased from the well differentiated to the slightly differentiated patterns.
- Regarding the possible mutual influences, the data provided by the statistical evaluation showed that:
  - The tumor stroma adapts to the arrangement of the population of tumor cells following the same arrangement with it, either by extension in an area type or by ordering in a linear model.
  - The intratumoral vascular network is also reconfigured according to the arrangement of the tumor cells, following the same architectural model as that of the cellular component, similar to the stromal component.
  - In contrast, there does not seem to be any influence or connection between the ways in which the two elements of the tumor cell population support structure are configured, the statistical apparatus proving that their evolutions towards one or the other arrangement model are independent from each other.

The **defining parameters of the biological behavior** of the tumor cell populations presented the same two categories of trends in the evolution of their expression

intensity, namely the evolution trends correlated with the degree of tumor differentiation and the trends of mutual influence.

- Regarding the correlation with the degree of differentiation, it was observed that:
  - The degree of intercellular adhesion showed a discrete tendency to decrease from the well differentiated to the weakly differentiated patterns, a tendency better expressed in the Srigley system.
  - The degradation capacity of the extracellular matrix is mainly provided by Gelatinase B/MMP9. Gelatinase A/MMP2 plays a secondary role, its secretion being almost two times lower than that of MMP9, independently of the type of tumor architecture. However, the expression values of the two matrix metalloproteinases do not seem to correlate with the degree of tumor differentiation, whether it is evaluated using the Gleason system or the Srigley system.
  - Neither did the level of tumor aggression, translated by the intensity of PTEN expression, show any variations in the degree of differentiation of the tumor proliferations, regardless of whether the evaluation system was Gleason or Srigley.
- Regarding the possible reciprocal influences, the data provided by the statistical evaluation showed the following aspects:
  - Intercellular adhesion, expressed by the intensity of ECAD immunostaining, was more pronounced in cell populations that showed a more pronounced expression of PTEN, in other words, a lower degree of aggression.
  - Paradoxically, the cell populations in which the cell adhesion was most pronounced produced a greater number of extracellular matrix degradation enzymes.
  - As a natural consequence of the aforementioned, it can be said that cell populations that produced more extracellular matrix degradation enzymes were less aggressive as they showed a more pronounced expression of PTEN.

Overall, the results suggest that:

- The tumor cell population models and adapts the stromal component and the surrounding vascular component in the same sense in which its architectural disposal evolves.
- The stromal component and the vascular network develop independently from each other, the first evolving towards the “area” architectural disposition model with the decrease of the degree of differentiation while the second evolves towards the “linear” architectural disposition model with the decrease of the degree differentiation.
- It appears that the classification system of the distribution arrangement of the tumor architecture proposed by Srigley, by grouping the subtypes described by Gleason, offers a more accurate description of the correlation between the tumor architecture and the degree of differentiation.
- The characteristic properties for the behavior of the evaluated malignant cell populations, namely the intercellular adhesion, the degradation capacity of the extracellular matrix and the degree of aggression, showed trends of synchronous evolution. Some of them seem somewhat natural, as in the case of decreased cellular adhesion in parallel with the decrease in PTEN expression. Others, however, are paradoxical, as is the case with the increase of the degradation capacity of the extracellular matrix in parallel with the increase of the degree of intercellular adhesion and with the decrease of the

degree of aggression (signaled by the PTEN overexpression). Of all, however, only the decrease in intercellular adhesion was correlated with the decrease in the degree of cell differentiation.

A final remark would be that the steps taken in this study must be continued on one hand by expanding the batches and on the other hand by introducing new parameters into the study and by extending the evaluation of the correlations between all the followed parameters.

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