Relationship of p27kip1 Expression to Incidence of Genetic Abnormities in Non-Generalised Prostate Carcinoma.

CYTOGENETICKÁ CYTOBIOPTICKÁ LABORATOŘ

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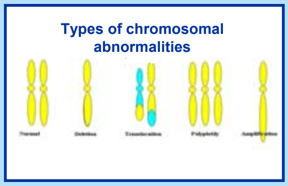
Goal of this Study

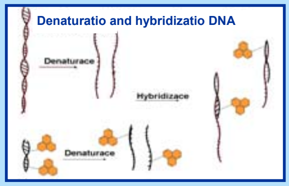
Formerly published studies concerning prostate carcinoma state that an amplification of the c-myc gene is firmly connected with an immunohistochemical over-expression of the Myc protein. There is an assumption that an over-expression of the Myc protein causes a degradation of the p27kip1 protein (hereinafter referred to as the p27) leading to an activation of the path of cyclin E/cyclin-dependent kinase 2 and cell proliferation. As it has been proven that many chromosomal and genetic abnormalities are associated with prostate carcinoma progression, the goal of this study was to prove whether there was a relationship of the p27 expression to those genetic abnormalities. As the chromosome 8 is the most frequently affected chromosome in prostate carcinoma, and the most frequently occurring chromosomal changes are 8p22 deletions and 8q24 region amplifications, these very changes were evaluated.

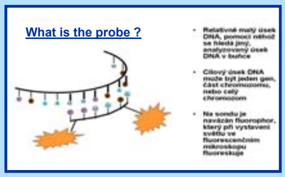
Material and Methods

From a group of 130 patients, in whom retrospective immunohistochemical examinations were conducted on their samples, in which non-metastasizing prostate carcinoma was primarily diagnosed, to compare AR expression with symptoms (markers) occurring in a regulation of cell cycle and apoptosis (5), a group of 48 patients was selected. That is a group of the people died within 5 years from the date on which the primary diagnosis was determined and the people surviving with Gleason score higher than 5. Out of the group of tumours, only 35 cases were hybridised successfully.

To determine amplification in the region of 8q24 or deletion in the region of 8p22, the method of fluorescence in situ hybridization (FISH) on paraffin sections was applied, with using a Pro Vysion Multi-Color Probe made by Vysis, Abbott, USA, which contained three differently marked sections of the chromosome 8 DNA.







FISH Procedure

Thin sections were deparaffinized, incubated in 0.2 M HCl at a laboratory temperature for 20 minutes, incubated in a solution of 1M NaSCN (sodium thiocyanate, 98-102% titration, Sigma, USA) at 80°C, predigested using protease (protease II,Vysis, Abbott, USA) at 37°C for 95 minutes, fixed in 10% buffered formalin at laboratory temperature fro 10 minutes and dried on a heating plate at 45-50 °C.

At the same time (in addition – není patrné, zda je slovem „současně" míněno „ve stejnou dobu", nebo „navíc"). Probes and DNA of the sample examined were denatured at a temperature of 85°C for 1 minute, and the samples were incubated for 8-24 hours at a temperature of 37°C in a humid chamber. After completion of the hybridization, the excess and non-hybridized probe was washed in 0.4x SSC (salt sodium citrate,Vysis, Abbott, USA) / 3% NP (nonylphenylpolyethylene glycol, Vysis, Abbott, USA) buffer at a temperature of 73°C for 2 minutes, the samples were rinsed in 2xSSC/0.1% NP buffer at laboratory temperature for 1 minute and, considering the unstable fluorochrome, dried in a dark place. After the cores were stained (counterstained, painted) using DAPI II (4,6diamidino-phenylindole, Vysis, Abbott, USA), the samples were covered with a cover glass. 50-100 separate cores of tumour cells were evaluated. For each core, the number of signals was determined for the region of 8p22, centromere of the chromosome 8 and the region of 8q24.

Evaluation (Rating)

Normal finding : < 10% of cells with 3 or more signals and < 55% of cells with one or 0 signal(s) for CEP 8.

Loss of CEP 8: > 55% of cells with 1 or 0 signal(s) for CE; Loss of 8p22: signal ratio for 8p22/CEP8 <0,85.

Loss of c-myc: signal ratio for c-myc/CEP8 < 0,9.

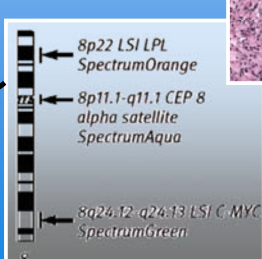
AI (additional increase): signal ratio fro c-myc/CEP8 >1.3 and >10% of cells with 3 or more signals fro c-myc.

AI = additional increase – that means an increase in the number of gene copies that is not accompanied with an adequate increase in the number of chromosomes

Expression of the p27 protein was evaluated as follows:

from 0 to 25 % of cells = significantly reduced expression

> 25% = normal expression.

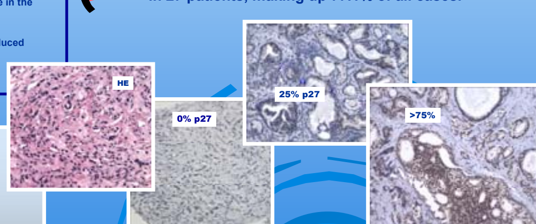


Results of thehybridization

p27 %positive nuclei	Group of Gleason score	Delece 8p22	Amplification 8q24	Polyzomia 8chromozom
15	2	no	yes (AI)	no(monozomie)
5	2	yes	no	no
0	2	yes	no	no
0	2	yes	no	no
25	3	yes	yes	yes
25	3	yes	yes	yes
5	2	yes	no	no
0	3	yes	yes	yes
25	2	yes	yes	yes
5	2	no	yes	yes
0	3	no	no	no
25	2	no	no	no
5	3	yes	yes	yes
50	3	yes	yes	yes
5	2	no	yes	yes
0	2	no	yes (AI)	no (monozomia)
0	2	no	no	no
25	2	yes	no	no
5	2	no	no	no
25	3	yes	yes	yes
0	2	yes	yes	yes
5	2	no	no	no
5	2	yes	no	no
5	2	yes	no	no
25	2	yes	no	no
5	2	no	no	no
0	1	yes	yes	no
0	1	no	no	no
25	3	yes	yes	yes
5	1	no	yes	yes
25	1	no	no	no
25	2	yes	yes	no
75	1	yes	yes	yes
0	2	no	yes	yes
0	2	yes	yes	yes

Results

Out of the total number of 48 hybridized samples, 35 cases were hybridized successfully, of which the cases where amplification in the region of 8q24 was observed were 19 (54.2%), however, in 15 of them (78.9%), polysomy of the chromosome 8 was proven at the same time. Deletion of the region of 8p22 was found in 21 cases (60%). Normal findings were proved in 8 cases (22.9%). Pathologic findings were recorded in 27 patients, making up 77.1% of all cases.



Acknowledgements

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Relationship of p27 Expression to Incidence of All Genetic Abnormalities Observed

% experession p27	With genetics abnormalit	without genetics abnormalit	summary
0-25%	25	8	33
	75,8%	24,2%	100,0%
50%	1	0	1
	100,0%	0,0%	100,0%
75%	1	0	1
	100,0%	0,0%	100,0%
Celkem	27	8	35
	77,1%	22,9%	100,0%

Degradation of the p27 represented by a low expression <25 is recorded in 78.5% of cases of all genetic abnormalities. However, it was also recorded in prostate carcinoma without any genetic abnormality occurrence recorded, exactly in 8 cases, corresponding to 24.2% of all degradations of the p27 protein expression. To perform statistical processing, the method of chi-square test was used. The resulting significance of the chi-square test is: $p = 0.226$. Although it is a value close to significance, the chi-square test, in statistical terms, has not proven a dependence of p27 expression on the incidence of genetic abnormalities.

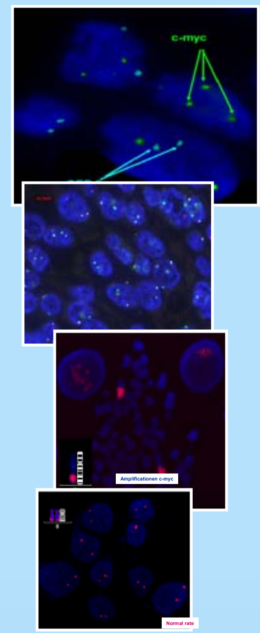
Results of the Relationship of p27 Expression to Incidence of 8p22 Deletion

% experession p27	deletion 8p22	without deletion 8p22	summary
0-25%	19	14	33
	57,6%	42,4%	100,0%
50%	1	0	1
	100,0%	0,0%	100,0%
75%	1	0	1
	100,0%	0,0%	100,0%
Celkem	21	14	35

Degradation of p27 expression occurs in 57.6% prostate carcinomas with an occurrence of 8p22 deletion and 42.4% of carcinomas without any occurrence of this genetic abnormity.

Chi-square test significance: $p = 0.503$.

The chi-square test has not proven a dependence of p27 expression on the incidence of the genetic abnormality consisting in 8p22 deletion.



Results of the Relationship of p27 Expression to Incidence of c-myc Amplification

% experession p27	Amplification c-myc	without amplification c-myc	summary
0-25%	17	16	33
	51,5%	48,5%	100,0%
50%	1	0	1
	100,0%	0,0%	100,0%
75%	1	0	1
	100,0%	0,0%	100,0%
Celkem	19	16	35
	54,3%	45,7%	100,0%

Out of the total number of 19 prostate carcinomas showing a c-myc amplification, 17 cases were recorded concurrently with decreased expression of the p27 (17 cases showed decreased expression of the p27 at the same time/as well), making up 89.5% of c-myc amplification carcinoma cases. The results are in accordance with data published in literature. Our study has proven that the incidence of amplification of the c-myc gene in prostate carcinomas causes degradation of p27 expression. However, the study also warns that a decreased expression of the p27 protein was also found in carcinomas without any amplification of the c-myc gene. The chi-square test significance is: $p = 0,409$.The relationship of p27 expression to the incidence of amplification of the c-myc gene is significant.

Conclusions

* **Conclusions**

Degradation of the p27 protein represented by an expression from 0 to 25% prevails in the group of carcinomas with chromosome change findings over the group without those changes. The ratio is 25 cases (75.8%) to 8 cases (24.2%). However, statistical processing has not proven a significance of the result, because the significance is 0.226. The main problem of the insignificance consists in the low frequency of cases in individual groups. The percentage of more then ¾ of chromosome aberrance cases in the group of degradation of the p27 protein shows an accord with cases published in literature.

* In carcinomas with amplification of the c-myc gene, a significantly decreased expression of the p27 protein was found in 17 cases, making up 89.5% of all prostate carcinomas with c-myc gene amplification. In 16 (48.5%) cases, however, amplification was not found. The unexpectedly numerous group of prostate carcinomas with a decreased expression of the p27 without amplification of the c-myc gene suggests an assumption on more possible manners of degradation of p27 expression. However, to prove this assumption, the group examined shall be extended substantially. The statistical processing using the chi–square test has proven a significant relationship of the dependence of degression of the p27 protein on amplification of the c-myc gene in prostate carcinoma. The probability is: $p=0.0409$

* In prostate carcinomas with 8p22 deletion, we had not expected a relationship to degradation of the p27 protein p27. The results correspond to that expectation: 19 (57.6%) cases with deletion to 14 (42,4%) cases without deletion.

The statistical results which are not in accordance with data published in literature may be distorted due to the low frequency of cases.

*Another problem may consist in the fact that the analysis was conducted on a rather low number of tumour cells. Sometimes, we could not meet the requirement for 300 tumour cores examined because of their relative lack in the puncture biopsy.

*Fixation of the material examined may be critical as well; therefore, we recommend that the fixation procedure (use of buffered formalin, determination of the fixation duration to at most 24 hours) be standardised in advance or samples after prostatectomy be used to ensure a sufficient amount of material.

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