Characterization of Bioenergetic Phenotype as Hallmark of Non-Muscle Invasive Bladder Cancer: Bioenergetic Signature of Low-Grade pTa, High-Grade pTa and pT1

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Keywords: Bladder cancer, Energy metabolism, Bioenergetic signature, Cancer metabolism

Background

To evaluate and understand how the neoplastic cells of each histological grade of the non-muscle-invasive bladder cancer (NMIBC) can undertake strategies of metabolic adaptation to attend the marked demands of cell growth and proliferation seems to be a field with promising perspectives in the best understanding of the behavior of these cells. **Therefore, the present study characterized and compared the profile of cellular energetic metabolism in the different histological grades of NMIBC (low-grade pTa, high-grade pTa, pT1), besides allowing the adaptation of the bioenergetic cellular index to these tumors.**

Methods

Thirty formalin-fixed paraffin-embedded samples of bladder were collected from patients with NMIBC who underwent transurethral resection and were divided into 3 groups (n= 10 samples per group): low-grade pTa group; high-grade pTa group and pT1 group. These samples were submitted to immunohistochemistry and Western Blotting analyzes: glycolytic pathway markers [Glucose transporter-1 (GLUT-1), Phosphofructokinase (PFK), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Lactate dehydrogenase A (LDH-A)] and; oxidative phosphorylation (OXPHOS) pathway [Pyruvate Dehydrogenase (PDH), Citrate synthase (CS), Short chain 3-hydroxyacyl-CoA dehydrogenase (HADHSC), Mitochondrial ATP synthase F1-beta-subunit (β-F1-ATP synthase) and heat shock protein 60 (HSP60)]. The bioenergetic cellular (BEC) index were calculated using β-F1-ATPase/HSP 60/GAPDH ratio.

Results

- Low-grade NMIBC (low-grade pTa group) showed significant difference ($p<0.01$) in energy metabolism in relation to high-grade NMIBC (high-grade pTa and pT1 groups);
- Low-grade tumors showed an avidity for the OXPHOS pathway, while the high-grade tumors by the glycolytic pathway;
- The low-grade pTa tumors had a high BEC index ($p<0.01$) because of the predominance of OXPHOS observed by intense immunoreactivity and higher-protein levels of β-F1-ATP synthase and, weak immunoreactivity and low-protein levels of GAPDH;
- Also, these tumors showed intense immunoreactivity and high-protein levels for HADHSC and PDH and, weak immunoreactivity and low-protein levels for GLUT-1 and PFK.

Table 1. Baseline Characteristics

	Low-grade pTa	High-grade pTa	pT1
Patients (n)	5/15	5/15	5/15
Man/Woman	3/2	3/2	4/1
Age (years) (median)	57	60	70
Smoking (yes/no)	4/1	4/1	4/1
Previous TURB (yes/no)	1/4	2/3	2/3
Previous BCG (yes/no)	0/5	0/5	1/4
Lesion size (median)	3.0 cm	3.0 cm	4.0 cm

Acknowledgements and Funding

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico · FAPESP · mm PHARMA

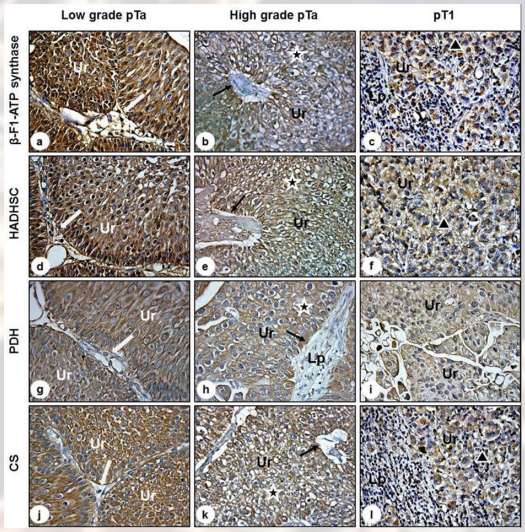

Figure 1a – 1l: Immunohistochemistry of the urinary bladders of Groups: Low-grade pTa (a, d, g and j), High-grade pTa (b, e, h and k) and pT1 (c, f, i and l). Cytoplasmic immunoreactivity for the protein levels of β-F1-ATP synthase (a, b and c), HADHSC (d, e and f), PDH (g, h and i) and CS (j, k and l) of urothelial cells. **Ur** - urothelium, **Lp** - lamina propria, **white arrow** - conjunctive-vascular axis, **black arrow** - basal membrane integrity, **star** - cellular atypia, **triangle** - cellular cords. Scale: 50μm.

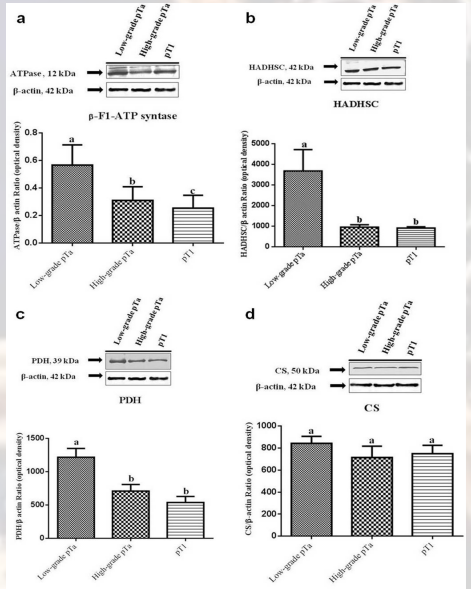

Figure 2a – 2d: Representative Western blotting and semi-quantitative determination for the protein levels of β-F1-ATP synthase (a), HADHSC (b), PDH (c) and CS (d). All data were expressed as mean ± standard deviation. Different lowercase letters (a, b, c) showed significant differences ($p<0.01$) between the groups after the Tukey test. Groups: low grade pTa, high grade pTa and pT1.

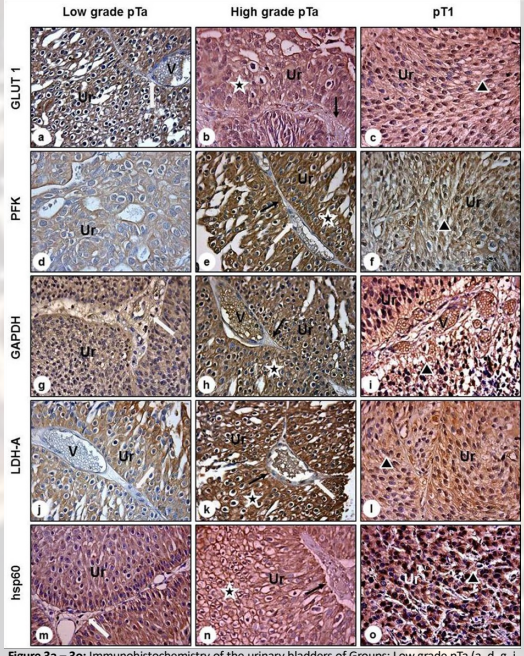

Figure 3a – 3o: Immunohistochemistry of the urinary bladders of Groups: Low grade pTa (a, d, g, j and m), High grade pTa (b, e, h, k and n) and pT1 (c, f, i, l and o). Cytoplasmic immunoreactivity for the protein levels of GLUT 1 (a, b and c), PFK (d, e and f), GAPDH (g, h and i), LDH-A (j, k and l) and hsp60 (m, n and o) of urothelial cells. **Ur** - urothelium, **V**- Blood vessel, **white arrow** - conjunctive-vascular axis, **black arrow** - basal membrane integrity, **star** - cellular atypia, **triangle** - cellular cords. Scale: 50μm.

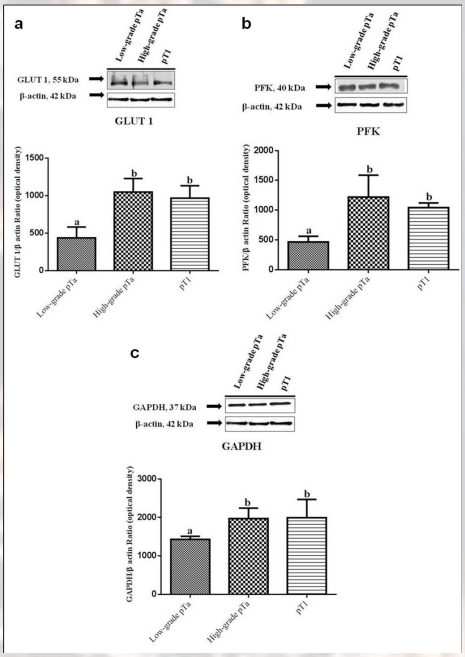

Figure 4a – 4c: Representative Western Blotting and semi-quantitative determination for the protein levels of GLUT 1 (a), PFK (b) and GAPDH (c). All data were expressed as mean ± standard deviation. Different lowercase letters (a, b) showed significant differences ($p<0.01$) between the groups after the Tukey test. Groups: Low grade pTa, high grade and pT1.

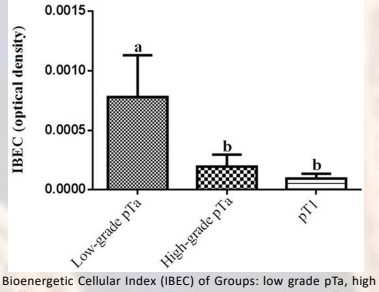

Figure 5: Bioenergetic Cellular Index (IBEC) of Groups: low grade pTa, high grade pTa and pT1. Different lowercase letters (a, b) showed significant differences ($p<0.01$) between the groups after the Tukey test.

Conclusions

The association between NMIBC with energetic cellular metabolism is of relevance in establishing clinical-pathological and prognostic criteria as well as helping to develop new therapies focused on the metabolism of these tumor cells that are more effective and with less side effects.