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Combined lectin- and immuno-histochemistry (CLIH) for applications in cell biology and cancer diagnosis: analysis of human urothelial carcinomas

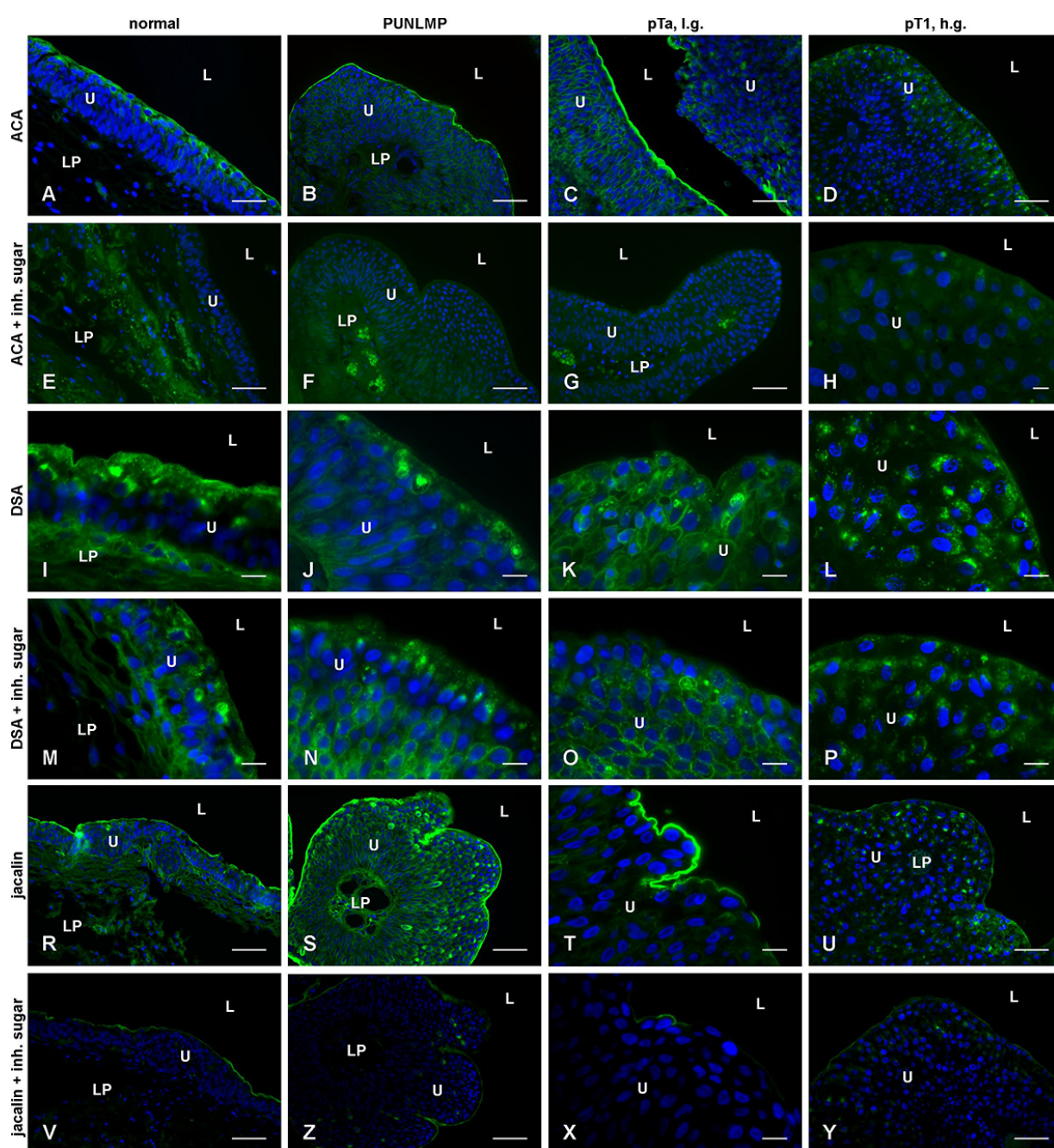
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Key words: lectin histochemistry; immunohistochemistry; combined lectin- and immuno-histochemistry (CLIH); paraffin sections; cryo-semithin sections; urothelium; papillary urothelial carcinoma.

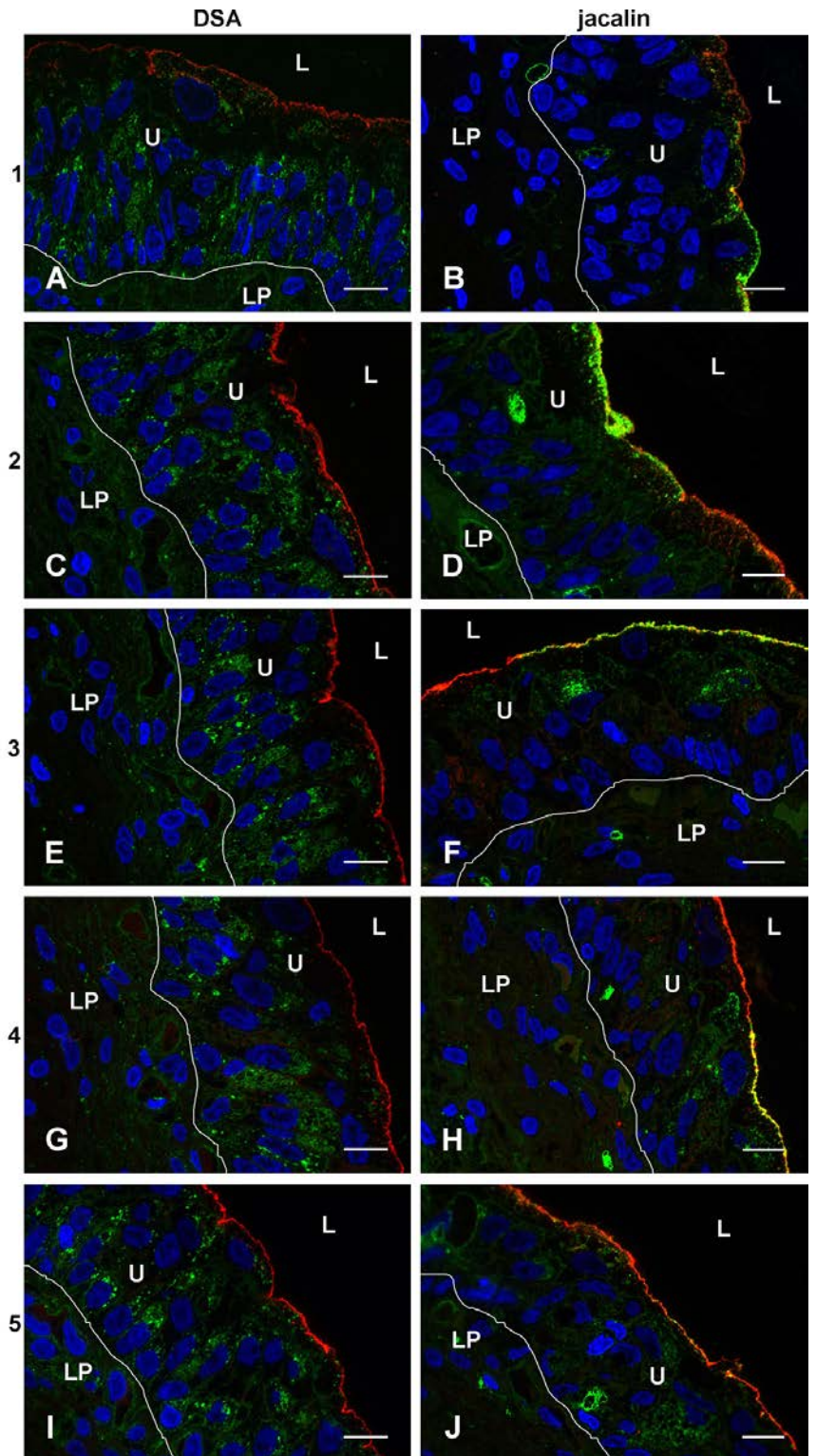


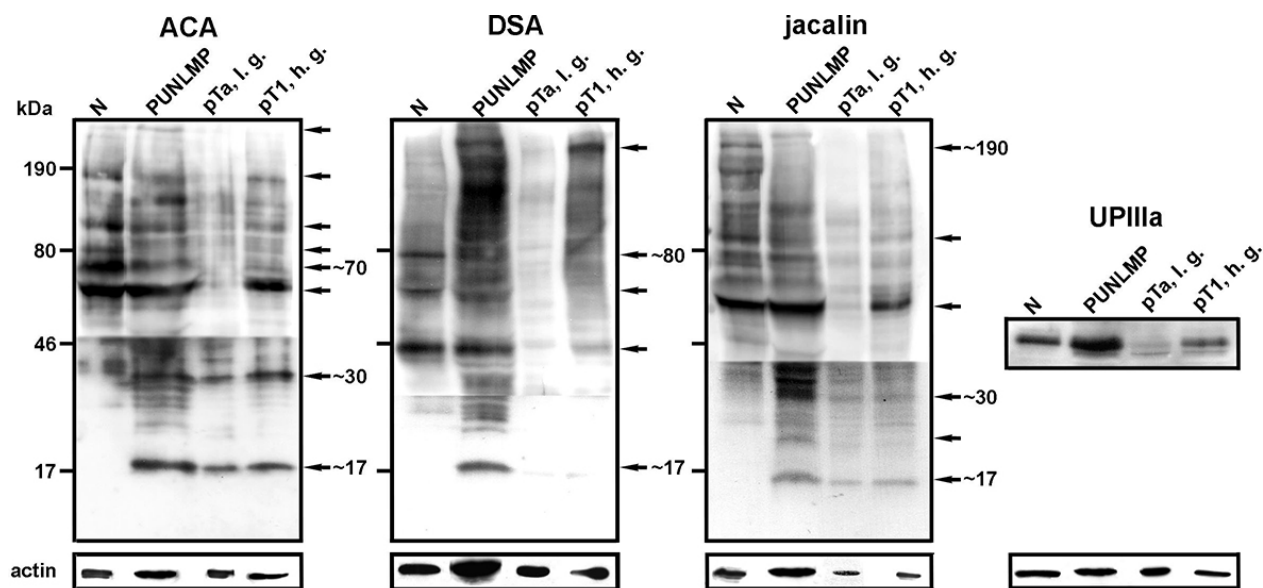
Supplementary Figure 1.

Lectin histochemistry on paraffin sections of normal urothelium and papillary carcinomas (PUNLMP, pTa, l.g., pT1, h.g.) without (ACA, DSA, jacalin) or with pre-incubation of the lectin with its inhibitory sugar (ACA+inh.sugar, DSA+inh.sugar, jacalin+inh.sugar). Scale bar: 50 μ m.

Supplementary Figure 2.

CLIH on cryo-semithin sections of normal human urothelium with antibodies against UPs (red) and lectins (green) DSA (A, C, E, G, I) and jacalin (B, D, F, H, J) following 5 protocols. There is no co-localisation between UPs and DSA, since UPs are present in the apical PM of umbrella cells, while DSA labelling is present in the cytoplasm of superficial, intermediate and basal cells. Co-localisation between UPs and jacalin labelling is present at some regions of the apical PM of umbrella cells (yellow), while other regions are only UPs positive (red) or only jacalin positive (green). White line outlines the basal lamina. L, lumen of the bladder; U, urothelium; LP, lamina propria. Scale bars: 10 μ m.





Supplementary Figure 3.

Lectin blotting with lectins ACA, DSA and jacalin in the samples of normal urothelium and different urothelial carcinomas (PUNLMP, pTa, l. g. and pT1, h. g.). Each blot is composed of two images from different membranes acquired from different separating gels (7.5% gel and 15% gel). Lectins bind to proteins of different molecular weights. The major differences between normal and carcinomas are marked by arrows. UPs expression determined by Western blotting in normal urothelium and different urothelial carcinomas (PUNLMP, pTa, l. g. and pT1, h. g.). Bands correspond to expected molecular weights (UPIIIa – 47 kDa). Note that in the sample of PUNLMP uroplakin expression is increased in comparison to normal urothelium, while in the samples of pTa, l.g. and pT1, h.g. it is decreased. Molecular weights are shown in kilodaltons (kDa).