

PROPATH LABORATORY

Immunohistochemistry Division

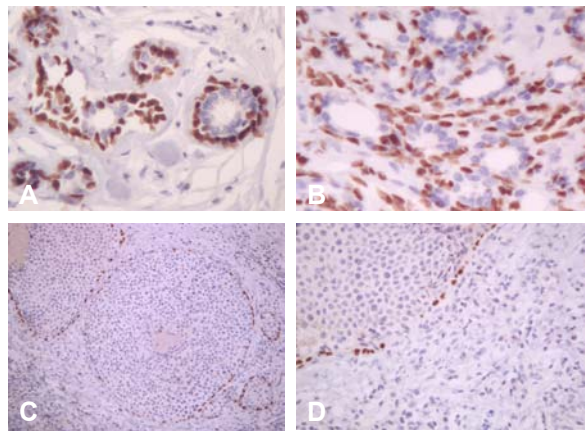
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Focus on Immunohistochemistry - September 2001 p63

Markers of myoepithelial cells and basal cells are extremely useful in diagnostic surgical pathology, particularly when examining difficult breast biopsies and prostate biopsies. In these situations, identification of an intact myoepithelial cell layer (in a breast biopsy) or an intact basal cell layer (in a prostate biopsy) can greatly assist in the distinction of carcinoma from benign mimics of malignancy. A recent article in the Aug. 2001 issue of the American Journal of Surgical Pathology called attention to a novel marker of myoepithelial cells, p63, that appears to have great promise in this area. p63 is a homologue of the p53 gene, and experimental studies have shown that p63 is necessary for normal breast and prostate development, and has also been found to be necessary to maintain stem cell epithelial populations in some tissues. Unlike other markers of myoepithelial cells and basal cells, p63 immunoreactivity is localized to the **nucleus** of the cells, which can offer distinct advantages over cytoplasmic labeling in certain types of cases.

Barbareschi and colleagues (1) studied a total of 384 samples of normal and pathologic breast tissues, including 300 invasive carcinomas and 20 cytologic specimens, as well as a number of frozen tissue samples. They found p63 to be a very sensitive and specific marker of myoepithelial cells in both normal and diseased breast tissue. As expected, benign breast lesions showed p63-positive myoepithelial cells, whereas invasive carcinomas lacked these cells (with the exception of adenoid cystic carcinoma, not surprising considering that this tumor shows myoepithelial differentiation). They also noted immunostaining in a small percentage of tumor cells (5-15%) in a small minority (<5%) of ductal carcinomas. P63 also highlighted the "naked nuclei" in fine needle aspiration biopsy specimens of fibroadenomas.

In the case of lobular carcinoma in situ and ductal carcinoma in situ, p63 showed a discontinuous rim of myoepithelial cells, similar to what is observed with other markers of myoepithelial cells.



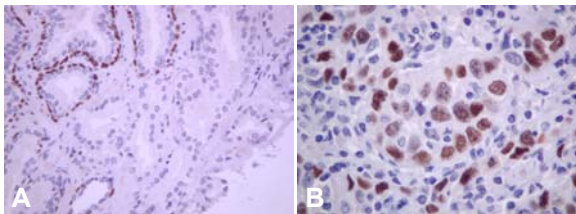
p63 immunostain on a normal breast lobule (A) shows nuclear staining of myoepithelial cells, also demonstrated nicely in a focus of sclerosing adenosis (B). In DCIS (C), there is a discontinuous layer present. Frame D shows a portion of a duct with DCIS on the upper left of this frame, bounded by p63-positive myoepithelial cells, with adjacent invasive carcinoma.

In particular, the authors noted that myofibroblasts were consistently negative for p63. Smooth muscle actin is used by some pathologists as a myoepithelial marker, but this marker also extensively stains myofibroblasts, severely limiting its utility in many cases. Calponin and smooth muscle myosin have greater specificity for myoepithelial cells and show much less staining of myofibroblasts, although on occasion these markers will also show myofibroblast staining. In these situations, the distinction of a myofibroblast from a myoepithelial cell may be very difficult, rendering interpretation of a problematic case difficult. However, with p63 this distinc-

tion should be much easier to make.

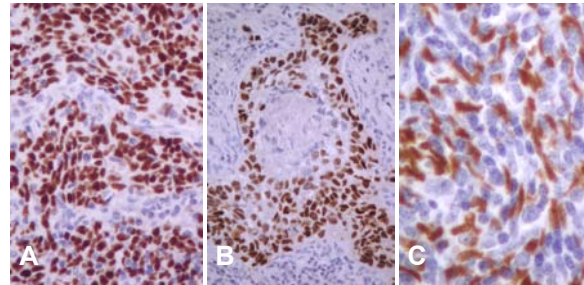
p63 has also been used as a marker of prostate basal cells, which to my knowledge was first reported by Signoretti et al (2, 3). Studies of prostate biopsy specimens at ProPath have confirmed their findings, and we have studied (and continue to study) problematic prostate needle biopsies with parallel stains for p63 and high molecular weight cytokeratin. At the time of this writing, we have found p63 to be as useful as high molecular weight cytokeratin, and there have been some cases where p63 has been easier to interpret than high molecular weight cytokeratin. We anticipate that these findings will be evaluated in other laboratories and eventually find their way into the mainstream diagnostic pathology literature in the not too distant future.

Studies with p63 using ProPath's multitumor tissue blocks (which contain 80 tumors of many different types) have also revealed some interesting findings, several of which were also previously reported at the March 2001 US-CAP meeting (4-5). Three of three squamous carcinomas (of uterine cervix, lung, and penis) present in the multitumor block were strongly positive. Obviously, this raises the possibility that p63 might serve as a marker of squamous differentiation, and indeed there are many similarities in the reactivity patterns of p63 and cytokeratin 5/6 (which is also a good squamous marker). (Parenthetically, as noted above, Barbareschi and colleagues noted p63 reactivity in two breast carcinomas that showed squamous metaplasia.) Four of four transitional cell carcinomas of the bladder studied at ProPath have also shown p63 immunoreactivity. Since these tumors do not express strong cytokeratin 5/6 (unless they are showing squamous differentiation), perhaps the p63 positive, CK 5/6 negative phenotype may be useful in recognizing transitional cell carcinoma, although obviously this will require further study. Additionally, if this observation is confirmed by additional study, p63 may



Prostate needle biopsy (A) shows intact p63-positive basal cells around the benign prostate glands (upper left of frame) with expected absent basal cells in the area of invasive carcinoma. Invasive transitional cell carcinoma of the bladder (B) shows strong nuclear staining with p63.

be a useful marker to distinguish cases of prostate carcinoma (p63-) from transitional cell carcinoma (p63+). We have also observed p63 reactivity in myoepithelial cells of submandibular gland, basal cells of respiratory epithelium, and in the epithelial cells of a spindle cell thymoma. Undoubtedly we will be reading more about this interesting new marker in the future.



p63 in invasive squamous carcinoma of uterine cervix (A) and lung (B). In the lung cancer, note that p63 staining decreases in intensity in areas of tumor keratinization (center of nest). Frame C shows p63 staining of spindled epithelial cells in thymoma.

P63 (clone 4A4) is now available in the ProPath Immunohistochemistry Laboratory, and although it is currently not listed on any of our immunohistochemistry request forms, it may be ordered by simply writing in "p63" on our request forms. Other recent antibodies that we have added to our list include **human placental lactogen (HPL)**, and **FLI-1** (although at the present time I have not been particularly impressed with the quality of the FLI-1 antibody, a not uncommon problem in our experience from this particular vendor).

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