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Technical note: p40 antibody as a replacement for p63 antibody in bovine mammary immunohistochemistry

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ABSTRACT

Tumor protein 63 (p63) is a nuclear antigen found in basal epithelial cells. To date, 10 isoforms of p63 have been identified, falling into 2 major groups identified by presence or absence of an N-terminal transactivation domain (TAp63 and ΔNp63, respectively). Literature suggests that ΔNp63 is the predominant form expressed in basal epithelial cells and myoepithelial cells (MYEC). The mouse anti-p63 antibody, clone 4B1E12, has been used as a specific nuclear marker for bovine MYEC. Unfortunately, this antibody is no longer commercially available. A new mouse monoclonal antibody, clone BC28, specific to ΔNp63 (designated p40) has been developed. We hypothesized that the p40 antibody would be an appropriate substitution as a MYEC and epithelial basal cell marker. An array of archived formalin-fixed, paraffin-embedded bovine tissues were subjected to immunohistochemical staining for either p40 or p63, with a subset being dual stained for direct comparison. Positive staining for p40 and p63 was observed in serial sections of mammary, skin, rumen, salivary gland, ureter, and bladder. As predicted, negative staining for p40 and p63 was observed in testis and intestine. Dual staining for p40 and p63 in calf mammary (n = 4), lactating mammary (n = 4), rumen (n = 4), and skin (n = 4) showed nearly 100% agreement. Thus, we established that the mouse monoclonal antibody, clone BC28, is a suitable replacement for anti-p63, clone 4B1E12, as a marker of MYEC and basal epithelial cells in bovine tissues.
Key words: mammary, myoepithelial, p63, p40

Technical Note

Tumor protein 63, also known as transformation-related protein 63 (**p63**), is a structural homolog of tumor protein p53. The gene has 2 promotors, which generate 2 N-terminal variants, one containing a transactivation domain (**TAp63**) and one without (**ΔNp63**). Additional splice variations give rise to 5 different C-termini (α, β, γ, δ, ε) for a total of 10 possible isoforms (Guerrini et al., 2011; Nobre et al., 2013). The TAp63 protein transactivates p53 target genes and induces apoptosis (Di Como et al., 2002), whereas ΔNp63 acts in a dominant negative manner to block transactivation. It is known that ΔNp63 also has specific transcriptional activities through a second small transactivation domain. (Browne et al., 2011; Guerrini et al., 2011; Nobre et al., 2013).

The importance of p63 in embryonic development is difficult to overstate. Mouse p63 double knock-out models demonstrated severe craniofacial, limb, and epithelial defects, with limb truncation and lack of epidermis. These animals died shortly after birth and demonstrated a lack of all squamous epithelia and derivatives, including mammary, lachrymal, and salivary glands (Yang et al., 1999). Thus, p63 protein serves a dual function: enabling terminal differentiation and stratification of epidermal cells by allowing cells to escape the stem state and cell cycle, and maintenance of the proliferative capacity of epidermal stem/progenitor cell populations (Guerrini et al., 2011; Nobre et al., 2013). Postnatal epidermal expression of p63 occurs in the basal nuclei of normal epithelia (skin, esophagus, tonsil, urogenital tract) as well as glandular epithelial structures such as salivary, mammary, and prostate glands (Guerrini et al., 2011; Nobre et al., 2013). Stratified epithelium expresses p63 basally and within 1 or 2 supra-basal cell layers (Hall et al., 2000). Basal cells predominantly express ΔNp63 (about 100-fold to TAp63), whereas cells in upper layers of stratified epithelia express TAp63 (Nobre et al., 2013).

Myoepithelial cells (**MYEC**) are located in the basal cell layer of normal mammary epithelia. Moreover, p63 has been found to be a selective nuclear marker of MYEC in the human breast (Barbareschi et al., 2001), alone or in combination with cytoplasmic markers such as smooth muscle myosin heavy chains, calponin, P-cadherin, mapsin, CD10, and smooth muscle actin (Barbareschi et al., 2001; Dewar et al., 2011). We have

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Table 1. Primary and secondary antibodies used in this experiment

Item	p63	p40
Primary antibody		
Host, serotype	Mouse, IgG_{2b}	Mouse, IgG_1
Source	No. 37–9500 Invitrogen, Waltham, MA	No. A21141 Abcam Inc., Cambridge, MA
Dilution	1:200	1:100
Secondary antibody		
Host, conjugate	Goat, Alexa 488	Goat, Alexa 594
Serotype	Anti- Mouse IgG_{2b}	Anti-Mouse IgG_1
Source	No. A21141 Thermo Fisher Scientific, Rockford, IL	No. A21125 Thermo Fisher Scientific
Dilution	1:200	1:200

used cytoplasmic markers smooth muscle actin and CD10, with or without p63, to identify and characterize MYEC in bovine mammary tissue (Safayi et al., 2012; Tucker et al., 2016). It is challenging to identify primary antibodies that are specific for, or cross-react successfully with, bovine antigens. Tucker et al. (2016) used a mouse anti-p63, clone 4B1E12 (No. 37–9500; Invitrogen, Waltham, MA) to label MYEC nuclei. This antibody was also successful in labeling basal cells of bovine skin and rumen epithelium (K. M. Daniels, unpublished data). Unfortunately, the manufacturer has discontinued this product.

Recently, human cancer diagnostic research has explored the use of primary antibodies specific to the ΔNp63 isoform (p40) versus traditionally used anti-p63 antibodies (such as clone 4A4), which recognize both TAp63 and ΔNp63 isoforms (Nobre et al., 2013; Tacha et al., 2014). We chose mouse monoclonal p40 (clone BC28) from Abcam (ab172731; Abcam Inc., Cambridge, MA) to compare with our remaining stock of mouse anti-p63 (clone 4B1E12) in identifying MYEC and other epithelial basal cell nuclei in an array of bovine tissues. We hypothesized that the p40 antibody would identify the same cell nuclei as the existing p63 antibody. Further, we hypothesized that the p40 antibody would improve specificity of basal cell identification in stratified epithelium, as ΔNp63 (p40) is suggested to be the dominant isoform in these cells (Nobre et al., 2013). According to the manufacturer (see Table 1), the p63 antibody was raised against a recombinant protein derived from the N-terminal region of human p63 (suggesting TAp63 or total p63), which would likely label more supra-basal epithelial cells.

Archived formalin-fixed, paraffin-embedded bovine tissues were used in this experiment. All tissues originated from animals enrolled in previously approved animal care and use protocols at Virginia Tech (DASC 14–045, 15–165). Samples of calf and lactating cow mammary parenchyma (n = 4), calf rumen (n = 4), and calf ear skin (n = 4) were dual immunofluorescently stained with p40 and p63 antibodies for direct comparison. Single samples representing other calf epithelial tissues were also stained with each antibody for presence or absence of labeled cells.

Table 2. Description of p63 and p40 in normal bovine tissues by immunohistochemistry

Organ site	p63 Score[1]	p40 Score[1]	Number of animals	Cells dual labeled[2] (% of total)
Digestive tract				
Rumen	(++)	(++)	4	97.4 ± 1.1
Intestine (ileum)	(-)	(-)	1	N/A[3]
Urinary system				
Bladder	(++)	(++)	1	N/A
Ureter	(++)	(++)	1	N/A
Reproductive system				
Mammary (calf)	(++)	(++)	4	98.7 ± 0.8
Mammary (lactating)	(+)	(+)	4	98.6 ± 3.2
Testis	(-)	(-)	1	N/A
Skin				
Epidermis	(++)	(++)	4	98.1 ± 0.7
Hair follicles	(++)	(++)		N/A
Sweat glands	(++)	(++)		N/A
Tonsil	(++)	(++)	1	N/A
Salivary gland	(++)	(++)	1	N/A

[1](-) = undetectable; (+) = moderate; (++) = strong.

[2]Data given as mean ± SEM.

[3]N/A = not quantified for % agreement.