Expression of p16 Protein in Cutaneous Basal Cell Carcinoma: Still far from Being Clearly Understood

Dear Editor,

$p16^{INK4a}$ protein (p16) is an important tumor suppressor protein involved in the carcinogenesis of many human malignancies. I have read with interest an article by Donati *et al.* (1) in this journal, who investigated an expression of p16 and proliferation marker Ki-67 in cutaneous tumors. Among them, there were 27 cases of non-melanoma skin cancer (NMSC), 23 of which comprised basal cell carcinoma (BCC), and 4 squamous cell carcinoma (SCC) lesions. The authors stated "in NMSC, they found a high prevalence (69.6%) of lesions with p16 expression between 34-66%, while the remaining specimens showed p16 expression ≤34%". Although they did not specify the exact proportions of BCC and SCC, one may speculate that the majority of BCCs (and maybe all) were at least partly immunoreactive for p16. As the literature gives very contradictory data on this topic, herein I will present my personal observations in this field.

I performed immunohistochemical analysis of p16 in a set of 24 cutaneous BCCs obtained from 23 patients. They were categorized into non-aggressive (4 superficial and 10 nodular subtypes) and aggressive (7 nodular-infiltrative and 3 infiltrative subtypes) subgroups. In all cases, monoclonal mouse antibody against p16 (clone G175-405, Zeta Corp., dilution 1:75) was used for staining. Overall, I found 7 cases (29.1%) of BCCs manifesting a certain degree of p16-immunoreactivity in the tumor tissue. These lesions arose on the head in four cases (4/17; 23.5%) and on the back and limbs in three cases (3/7; 42.8%). Histologically, they comprised four cases (4/14; 28.6%) of non-aggressive and three cases (3/10; 30%) of aggressive histologic subtypes, respectively. As regards to the extent of p16-positivity, it was only focal and involved merely 5-20% of total cancer tissue. Notably, p16-positive areas occurred only at the edges and at the invasive margins of tumor aggregates (Figure 1), except for the case of pure infiltrative BCC subtype in which they were haphazardly distributed within the tumor mass (Figure 2). p16-reactivity was not observed at the center of solid tumor nests.

Immunohistochemical expression of p16 in cutaneous BCC is very variable and reported values vary greatly between publications. Some authors (2-6) demonstrated it in the vast majority (79.2-100%) of BCCs. Conscience *et al.* (7) and Zheng *et al.* (8)

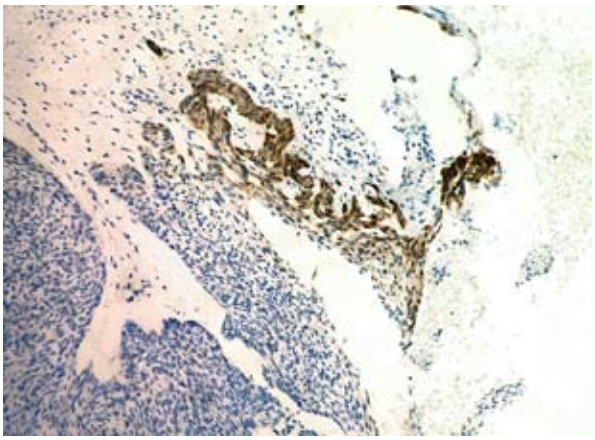

Figure 1. Cytoplasmic positivity for p16 (brown color) in nodular BCC at the edge of the tumor nest (magnification ×20).

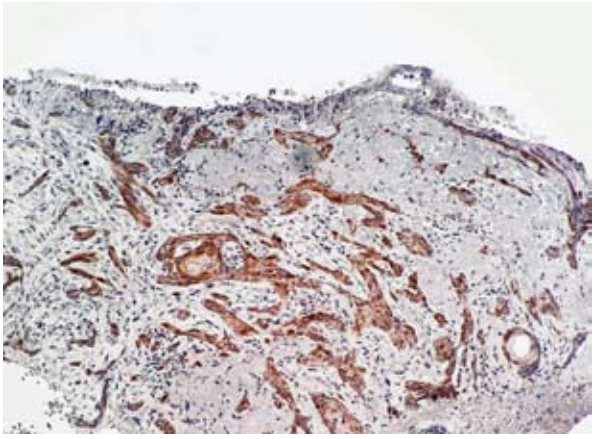

Figure 2. An area of infiltrative BCC manifesting diffuse cytoplasmic reactivity for p16 (brown color) (magnification ×20).

observed it only in 50% and 14.9% of the cases, respectively. Finally, Villada *et al.* (9) did not report any p16-positive BCC in their recent study. Based on the literature review (1-9), I speculate that such striking discrepancies could be attributed to the following reasons: a) different processing technique and methods used; b) heterogeneity of the biopsy sample size; c) case selection bias in terms of the prevalence of certain histological BCC subtypes; and d) different cut-off values defining the p16-positivity of tumors.

Biologic and prognostic impact of p16 production in cutaneous BCC remains unclear. In principle, it would be valuable to know whether BCCs with at least focal p16 immunostaining behave differently from their p16-negative counterparts. Several studies have reported conflicting data regarding a potential effect of p16 to invasive properties. Svensson et al. (5) showed that p16 expression was associated with invasive BCCs with an infiltrative growth pattern. In superficial, nodular, and infiltrative histologic subtypes, they found p16-positivity in 75.0%, 88.8%, and 100.0% of the cases, respectively. Other authors (2,3) did not find a clear relationship between the aggressive growth phenotype of BCC and immunoreactivity for p16. The data presented herein support the results of the latter group, but the present sample of p16-positive BCCs was too small to provide a reliable conclusion. Nevertheless, evidence of p16-immunoreactivity at the edges of tumor nodules and in the infiltrative growth pattern was similar with the findings published by Svensson *et al.* (5).

Another useful question is whether p16 protein production in neoplastic cells depends on the topographic distribution of lesions and if it may thus be influenced by solar exposure. Some authors (1,3,7) found that p16 overexpression was associated with NMSC arising on sun-exposed areas, suggesting a possible induction of p16 protein production by permanent ultraviolet radiation. On the other hand, Italian researchers (4) did not find such an association, as among the five BCCs arising on the head and neck region only one displayed a high p16 immunoreactivity. Furthermore, in the study conducted by Villada *et al.* (9), all ten p16-negative BCCs were situated on the head and neck.

Taken together, the biologic and clinical aspects of p16 production in cutaneous BCC are still far from being clearly understood. I assume that a simple quantification of p16 expression in BCC by immunohistochemistry is not sufficient for a reliable assessment of the clinicopathologic significances. Further studies must be more focused on spatial distribution and intensity of p16-positive areas in tumor tissue.

References:

1. Donati P, Paolino G, Panetta C, Cota C, Zaccarini M, Muscardin L. Another point of view on p16 and Ki67 expression in melanocytic and non-melanocytic cutaneous lesions. Acta Dermatovenerol Croat. 2013;21:149-54.
2. Ramezani M, Abdali E, Khazaei S, Vaisi-Raygani A, Sadeghi M. P16INK4a immunostaining but lack of human papilloma virus type 16 in cutaneous squamous cell carcinoma and basal cell carcinoma: a report from West Iran. Asian Pac J Cancer Prev. 2016;17:1093-6.
3. Cretnik M, Poje G, Musani V, Kruslin B, Ozretic P, Tomas D, *et al.* Involvement of p16 and PTCH in pathogenesis of melanoma and basal cell carcinoma. Int J Oncol. 2009;34:1045-50.
4. Paolini F, Carbone A, Benevolo M, Silipo V, Rollo F, Covello R, *et al.* Human Papillomaviruses, p16INK4a and Akt expression in basal cell carcinoma. J Exp Clin Canc Res. 2011;30:108.
5. Svensson S, Nilsson K, Ringberg A, Landberg G. Invade or proliferate? Two contrasting events in malignant behavior governed by $p16^{INK4a}$ and an intact Rb pathway illustrated by a model system of basal cell carcinoma. Cancer Res. 2003;63:1737-42.
6. Eshkoor SA, Ismail P, Rahman SA, Mirinargesi M, Oshkour SA. Immunohistochemical expression of CCND1 and p16 genes in tissue microarray. Austral-Asian J Cancer. 2009;8:103-8.
7. Conscience I, Jovenin N, Coissard C, Lorenzato M, Durlach A, Grange F, et al. P16 is overexpressed in cutaneous carcinomas located on sun-exposed areas. Eur J Dermatol. 2006;16:518-22.
8. Zheng Z, Kye Y, Zhang X, Kim A, Kim I. Expression of p63, bcl-2, bcl-6 and p16 in basal cell carcinoma and squamous cell carcinoma of the skin. Korean J Pathol. 2005;39:91-8.
9. Villada G, Kryvenko ON, Campuzano-Zuluaga G, Kovacs C, Chapman J, Gomez-Fernandez C. A limited immunohistochemical panel to distinguish basal cell carcinoma of cutaneous origin from basaloid squamous cell carcinoma of the head and neck. Appl Immunohistochem Mol Morphol. 2018;26:126-31.

Vladimir Bartoš, MD

Faculty Hospital, Žilina, Slovakia

vladim.bartos@gmail.com