

any histopathological evidence of thrombosis, and thus the diagnosis of injection-site reaction of 5-azacitidine was made based on a combination of clinical and histopathological findings. Most injection-site erythema/reactions are resolved by simple continuing treatment, and less than 12% of cases require corticosteroids and/or antihistamine². A correct injection technique, such as syringe aspiration, and injection-site rotation, can help prevent injection-site reaction.

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Upregulated Expression of Calcyclin-Binding Protein/Siah-1 Interacting Protein in Malignant Melanoma

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Dear Editor:

The calcyclin-binding protein (CacyBP) was initially named for its ability to interact with calcyclin (S100A6) at a physiological range of Ca²⁺ concentration¹. However, Matsuzawa and Reed² found that the human analog of mouse CacyBP interacted with Siah-1 and named this protein the Siah-1 interacting protein (SIP); therefore, it is

now widely called CacyBP/SIP. Additionally, CacyBP/SIP and Siah-1 associate with Skp-1, acting as an ubiquitinating complex that degrades non-phosphorylated β -catenin in the presence of p53².

In breast cancer, CacyBP/SIP mRNA and protein levels were significantly higher than that of adjacent non-tumor tissues. Poor cellular differentiation, lymph node invasion,

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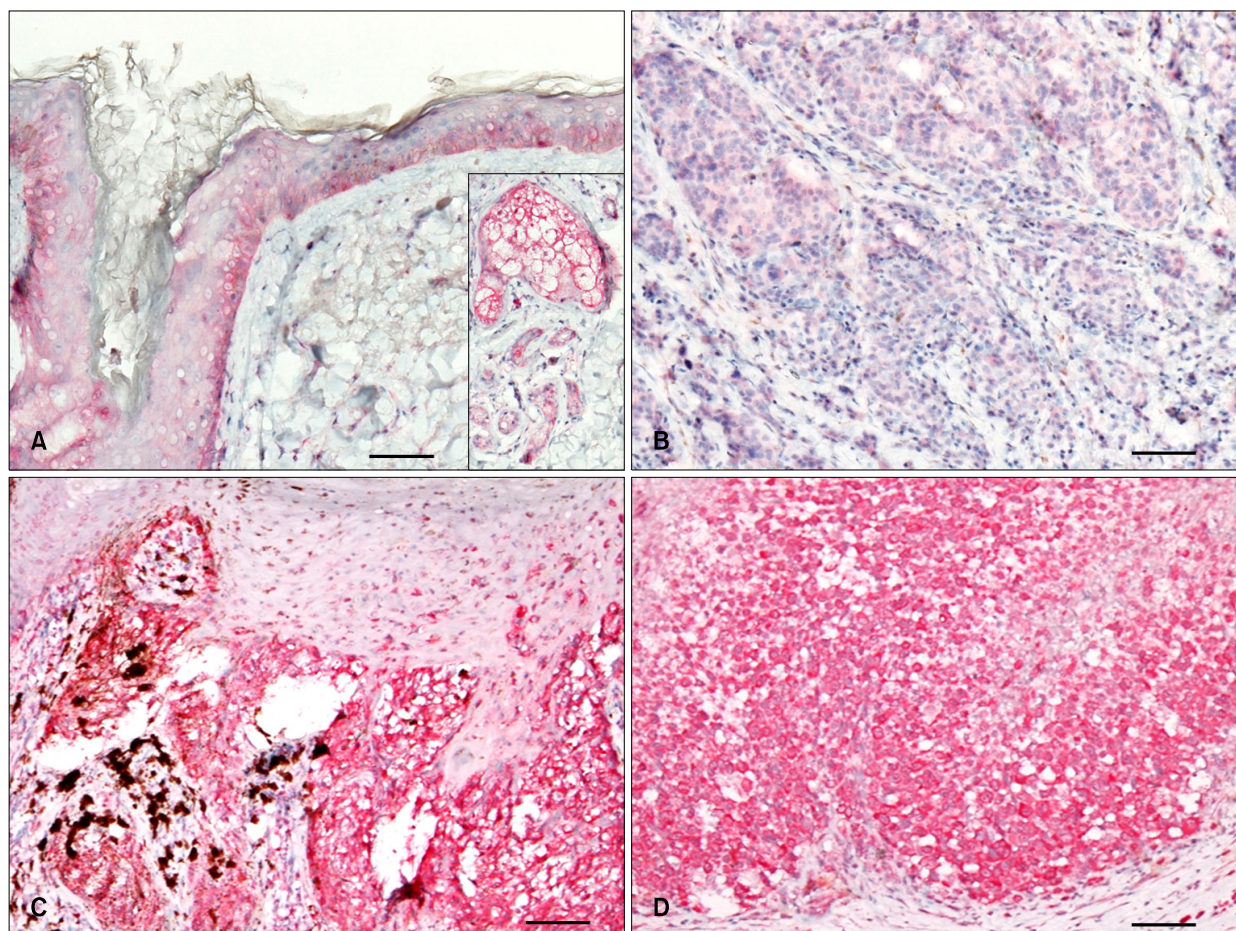


Fig. 1. Expression of calcyclin-binding protein/Siah-1 interacting protein in (A) normal skin; sebaceous and glands were depicted in the box, (B) benign melanocytic nevus, (C) primary malignant melanoma (MM), and (D) metastatic MM. Scale bar=100 μ m.

and clinicopathological staging in breast cancer were associated with CacyBP/SIP expression³, with similar findings in pancreatic cancer⁴. Although initially identified as a binding protein of S100A6, CacyBP/SIP has demonstrated an ability to bind with other S100 proteins such as S100B, S100P, S100A1, and S100A12⁵.

Widely and routinely used for immunohistochemical detection of malignant melanoma (MM), protein S100 and the upregulated expression of S100A6 were correlated with unfavorable prognoses of MM⁶⁻⁸; however, the expression levels of CacyBP/SIP have not been investigated. In this study, we compared the immunohistological expression of CacyBP/SIP in 20 primary MM, 20 metastatic MM, 20 benign melanocytic nevus (BN), and 10 normal skin samples.

Paraffin-embedded sections (4- μ m in thickness) were deparaffinized with xylene for 10 min and rehydrated through a graded ethanol series. Antibody-binding epitopes were retrieved by pressure-cooking the tissue sections in 10 mM/L sodium citrate buffer (pH 7.0; Yatoro, Tokyo,

Japan) for 10 min and the nonspecific binding was blocked using 10% normal rabbit serum (Novus Biologicals, Littleton, CO, USA). The sections were then incubated with an antibody against CacyBP/SIP (1 : 200; Novus Biologicals, Littleton, CO, USA) at 4°C overnight. Immunodetection was conducted using a standard streptavidin-biotin amplification method with 3-amino-9-ethylcarbazole as a chromogen followed by light counterstaining with hematoxylin. In each specimen, 3 high-power fields (HPFs, $\times 200$) of strong reaction were randomly selected, 100 tumor cells were counted in each field, and the average percentage of positively stained cells in each of the 3 HPFs was computed for each sample. We also evaluated the staining intensity of the specimens using the staining intensity of sebaceous glands as internal positive control. The staining intensity was semiquantitatively classified as negative, mild, moderate, and strong. The results of the one-way ANOVA were considered statistically significant at $p < 0.05$.

In normal skin, weak expression of CacyBP/SIP was

Table 1. Expression of CacyBP/SIP in MM and BN

	BN (n=20)	Primary MM (n=20)	Metastatic MM (n=20)
Percent expression (%)			
0	0	0	0
1~25	7	1	0
26~50	3	3	0
>50	10	16	20
Intensity of staining			
Negative	0	0	0
Weak	10	1	0
Moderate	7	5	1
Strong	3	14	19

After immunohistochemical staining, 3 high-power field images (HPFs, $\times 200$) were randomly selected in each specimen, 100 tumor cells were counted in each field, and the mean percentage of positively stained cells from the 3 HPFs was calculated. Percent expression was graded semiquantitatively as 0%, 1~25%, 26~50% or >50% of the tumor cells were stained. Intensity of staining was graded semiquantitatively as negative, weak, moderate, or strong, respectively. CacyBP: calcyclin-binding protein, SIP: Siah-1 interacting protein, MM: malignant melanoma, BN: benign melanocytic nevus.

detected along the basal epidermis. Strong expression was observed in the sebaceous glands with weak to moderate staining in hair follicle and eccrine glands (Fig. 1A). The BN cells generally expressed low amounts of CacyBP/SIP with 48.16 ± 6.639 percent positivity (Table 1, Fig. 1B), suggesting that there was no difference between the staining of junctional and intradermal components. However, the staining intensity of primary and metastatic MM was mostly moderate to strong, which was significantly stronger than that of BN ($p < 0.05$) (Table 1, Fig. 1C, D). Further, the percent positivity of CacyBP/SIP in primary and metastatic MMs were 74.00 ± 5.674 and 90.71 ± 2.001 , respectively, again significantly higher than that of BN (primary MM vs. BN, $p < 0.05$; metastatic MM vs. BN, $p < 0.01$). Moreover, the expression levels of CacyBP/SIP in metastatic MMs were significantly higher than those of primary MMs ($p < 0.05$).

Because S100A2, S100A6, S100A7, and S100P are variably and spatiotemporally expressed in the epidermis and skin appendages^{9,10}, the S100-binding protein and CacyBP/SIP were expected to be present in the skin samples. We first demonstrated the immunohistological localization of CacyBP/SIP in normal epidermis, hair follicle, sebaceous gland, and eccrine gland suggesting a physiologic role of S100-CacyBP/SIP in maintaining the epidermal and appendageal homeostasis. It has been reported that S100A6 is expressed in melanocytic lesions⁶ and is significantly correlated with the depth of invasion (Clark levels)⁷. Moreover, since the upregulation of CacyBP/SIP was also

documented in pancreatic cancers⁴, we speculated the overexpression of CacyBP/SIP in MMs, which was the case. The expression levels of CacyBP/SIP were, in fact, significantly higher than those of BN, with the metastatic MM exhibiting higher expression of CacyBP/SIP than the primary MM.

Although the biological significance of its upregulation remains unknown, CacyBP/SIP enhances the polyubiquitination and degradation of β -catenin, possibly accelerating melanoma progression by inhibiting β -catenin-mediated apoptosis².

In conclusion, the upregulated expression of CacyBP/SIP may potentially be related to the melanoma progression; therefore, further studies are needed to elucidate a functional role of CacyBP/SIP.

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