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ORIGINAL ARTICLE

Expression of fatty acid synthase (FASN) in oral nevi and melanoma

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OBJECTIVE: The aim of this study was to determine the expression of fatty acid synthase (FASN) in oral nevi and melanomas, comparing the results with correspondent cutaneous lesions.

MATERIALS AND METHODS: Expression of FASN was evaluated by immunohistochemistry in 51 oral melanocytic lesions, including 38 intramucosal nevi and 13 primary oral melanomas, in 10 cutaneous nevi and in 14 melanomas.

RESULTS: Fatty acid synthase was strongly expressed only in melanomas, either of the oral mucosa or cutaneous. On the other hand, most oral and cutaneous nevi were negative, with a few oral cases showing focal and weak expression.

CONCLUSION: Fatty acid synthase is expressed in malignant melanocytes, and it can be a helpful marker to distinguish oral melanomas from oral melanocytic nevi.
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Keywords: melanocytic nevi; melanoma; fatty acid synthase; immunohistochemistry; mouth

Introduction

Fatty acid synthase (FASN) is a multifunctional enzyme that participates in the endogenous synthesis of saturated long-chain fatty acid, from the small carbon precursors acetyl-CoA and malonyl-CoA (Jayakumar *et al*, 1995; Baron *et al*, 2004). FASN is downregulated in most normal cells, except in lipogenic tissues such as liver, sebaceous gland, lactating breast, fetal lung, and adipose tissue, because most of the fatty acids are supplied by the diet (Weiss *et al*, 1986; Kuhajda, 2000). On the other hand, FASN expression is upregulated in several human cancers including prostate, breast, ovarian, lung, stomach, colon, bladder, oral squamous cell carcinoma, cutaneous melanomas, and soft tissue sarcomas (Kusakabe *et al*, 2002; Swinnen *et al*, 2002; Rossi *et al*, 2003; Takahiro *et al*, 2003; Silva *et al*, 2004, 2008; Kapur *et al*, 2005; Carvalho *et al*, 2008) FASN overexpression has also been suggested as a potential prognostic factor associated with a poor prognosis, increased risk of recurrence, and metastases (Shurbaji *et al*, 1996; Gansler *et al*, 1997; Innocenzi *et al*, 2003). Over 90% of melanomas occur in the skin, but they may also affect less frequently the oral mucosa, esophagus, meninges, and the eyes (Prasad *et al*, 2004; Femiano *et al*, 2008). In cutaneous melanomas, FASN protein expression has been associated with Breslow thickness and consequently with a poorer prognosis (Innocenzi *et al*, 2003; Kapur *et al*, 2005). Nevertheless, there are no data about the expression of FASN in oral nevi and melanoma.

The objective of this work was to determine the expression of FASN in oral nevi and melanomas, comparing the results with correspondent cutaneous lesions.

Materials and methods

Formalin-fixed, paraffin-embedded tissue blocks were obtained from 51 and 24 oral and cutaneous melanocytic lesions, respectively. Oral lesions corresponded to 38 intramucosal nevi and 13 primary oral melanomas, and cutaneous included 10 compound melanocytic nevi and 14 melanomas (11 superficial spreading melanomas and three nodular). All lesions were revised using H&E preparations to confirm the diagnosis. Primary oral melanomas were classified according to Prasad *et al* (2004), and 11 and two cases corresponded to level III (very deep invasion) and *in situ*, respectively.

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Cutaneous melanomas were histologically classified using Breslow thickness and Clark level index (Breslow, 1970). Five cases had a Breslow thickness <1 mm, six cases between 1 and 2 mm, and three cases >2 mm. One case had Clark level I, 10 cases level II, and three cases level III.

For immunohistochemical staining, 3-μm-thick sections mounted on silane-coated glass slides were used. Briefly, the sections were deparaffinized and rehydrated in graded ethanol solutions, and after antigen retrieval with EDTA/Tris buffer (pH 9.0) in a microwave oven (1380 W; Panasonic, São Paulo, Brazil), endogenous peroxidase activity was blocked with 20% H_2O_2 by five cycles of 5 min each. Overnight incubation with the primary antibody anti-FASN (BD Biosciences, Franklin Lakes, NJ, USA) diluted in bovine serum albumin (1:200) was followed by the incubation with secondary antibody conjugated with polymer dextran marked with peroxidase (Dako EnVision Labelled Polymer; Dako, Glostrup, Denmark). The reaction was developed with Permanent Red (Permanent Red Substrate System; Dako) and counterstained with Carazzi hematoxylin. Positive and negative controls were included in all reactions.

Fatty acid synthase was analyzed using a combined scoring system based on both the fraction of positive tumor cells and the predominant staining intensity in the tumor according to Innocenzi *et al* (2003). The fraction of positive cells was estimated using a four-tiered scale ($<10\% = 1$, $11–50\% = 2$, $51–80\% = 3$, and $>80\% = 4$). The staining intensity was graded subjectively from 0 to 3 (negative: 0, low intensity: 1, moderate: 2, and strong: 3).

Results

The demographic data of the 75 oral and cutaneous melanocytic lesions are summarized in Table 1. All oral melanomas either *in situ* or invasive showed strong staining intensity for FASN antibody in more than 80% of malignant cells, while 30 cases of oral melanocytic nevi were negative and eight expressed the protein focally ($<10\%$) with low intensity. Epithelium of the normal mucosa was negative on the basal layer and diffusely positive in the cytoplasm of cells of the stratum spinosum and granulosum (Figure 1).

Considering the cutaneous lesions, FASN protein was strongly expressed in more than 80% of neoplastic cells in all cases of melanomas and negative for all compound melanocytic nevi (Figure 2). In melanomas, staining was more intense in cases with Clark level III and Breslow thickness >2 mm (three cases with strong staining)

Table 1 Demographic data of 75 oral and cutaneous melanocytic lesions

Tumor type	*Number of cases*	*Age range (mean years)*	*Male*	*Female*	*Localization*
Intramucosal nevi	38	16–67 (36)	8	30	Hard palate (13), buccal mucosa (11), gingiva (10), not specified (4)
Primary oral melanoma	13	23–86 (58)	4	9	Hard palate (8), hard palate and upper gingiva (3), upper gingiva (2)
Compound melanocytic nevi	10	18–58 (38)	6	9	Upper extremity (3), lower extremity (2), head and neck (1), trunk (3), not specified (1).
Cutaneous melanoma	14	31–75 (58)	3	11	Upper extremity (3), lower extremity (2), head and neck (4), trunk (5)

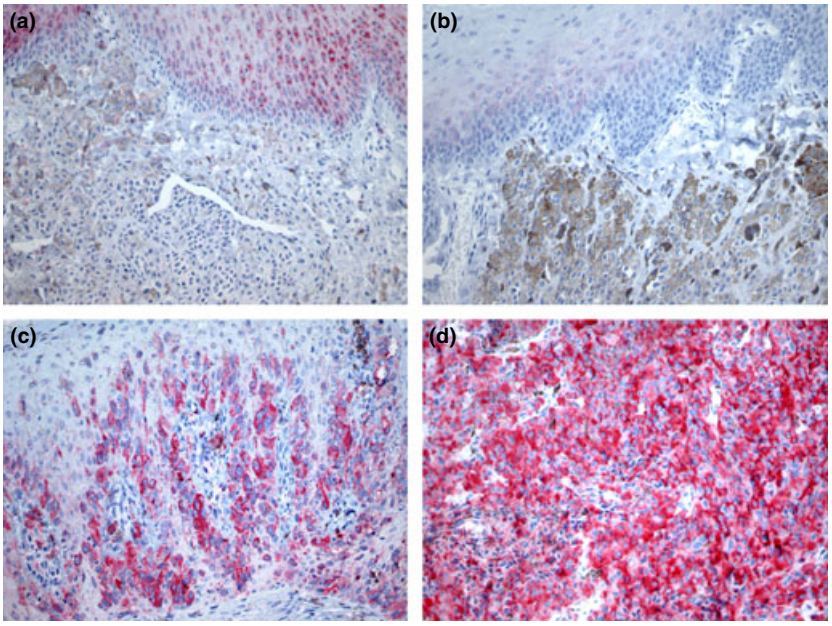

Figure 1 Expression of fatty acid synthase (-FASN) in oral melanocytic lesions: (**a**) intramucosal nevi showing FASN expression in the stratum spinosum of normal oral epithelium, while the nevi is negative, (**b**) intramucosal nevi rich in melanin showing absence of FASN expression, (**c**) oral *in situ* melanoma showing positivity for FASN, (**d**) invasive oral melanoma demonstrating high expression of FASN (Envision-Permanent Red, **A**–**D**, ×200)

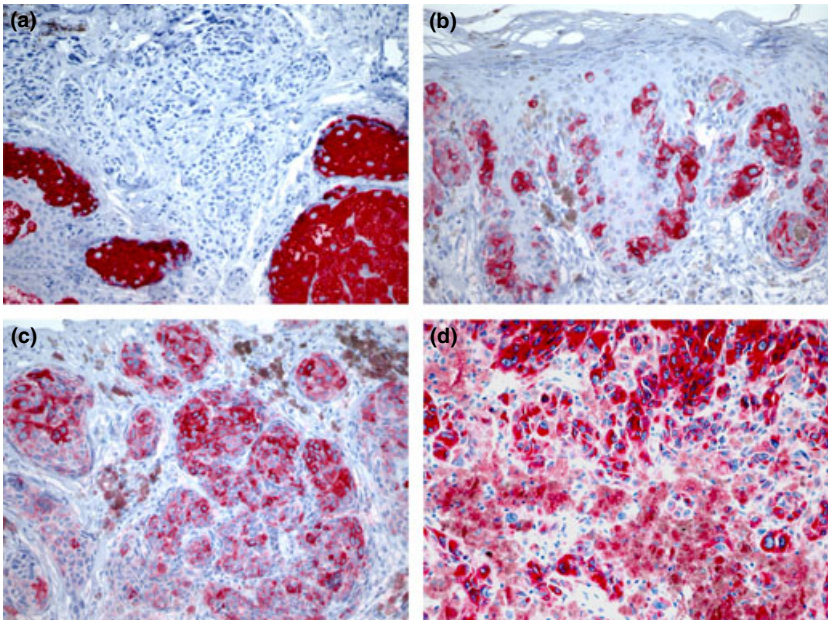

Figure 2 Expression of fatty acid synthase (FASN) in cutaneous melanocytic lesions: (**a**) compound melanocytic nevi which demonstrates strong FASN positivity only in normal sebaceous glands, (**b**) expression of FASN in Clark I melanoma cells, (**c**) Clark II melanoma with melanocytic cells positive for FASN, (**d**) strong FASN expression in neoplastic cells of Clark III melanoma (Envision-Permanent Red, **a**–**d**, ×200)

when compared with melanomas Clark levels I and II and Breslow thickness <2 mm (11 cases with moderate staining). Normal skin epithelium was negative for FASN, except the sebaceous gland that strongly expressed this protein (Figure 2).

Discussion

Fatty acid synthase is the key enzyme responsible for the synthesis of fatty acids, catalyzing the conversion of acetyl-CoA and malonyl-CoA into long-chain fatty acids (Jayakumar *et al*, 1995; Baron *et al*, 2004). Normal cells do not express FASN because they use fatty acids from dietary lipids, except those that are lipogenic (Kusakabe *et al*, 2000). Nevertheless, it is abnormally expressed in many human cancers cells because of their increased energy need (Visca *et al*, 2003; da Silva *et al*, 2009).

The regulation of FASN production in normal and malignant cells is complex and not well understood. Progesterone stimulates FASN expression in breast cancer cells lines (Lacasa *et al*, 2001), while in prostate cancer, FASN activity is upregulated by androgens and epidermal growth factor (Heemers *et al*, 2001). It seems that FASN is essential for cancer cell survival, as its specific inhibitors like cerulenin, C75, or Orlistat reduce cell proliferation and stimulate apoptosis (Li *et al*, 2001; Zhou *et al*, 2003; Kridel *et al*, 2004; Alli *et al*, 2005; Carvalho *et al*, 2008). These findings indicate that activation of fatty acid synthesis may be required for the high proliferation levels of cancer cells (Silva *et al*, 2004).

There are no data on expression of FASN in oral melanocytic lesions, and only two reports consider its relevance in cutaneous melanomas and nevi (Innocenzi *et al*, 2003; Kapur *et al*, 2005). We found that FASN was strongly expressed in all oral melanomas studied, and it was negative or eventually focally and weakly expressed in eight of 38 cases of oral nevi. Therefore, FASN activation in oral melanomas, as in various other cancers, seems to be relevant for malignant transformation of oral melanocytes. Also, it can be potentially useful as a diagnostic marker to distinguish nevi from melanoma. In fact, two cases of *in situ* oral melanomas were also strongly positive for this protein. Surprisingly, Kapur *et al* (2005) demonstrated that congenital nevi show high levels of fatty acid synthase expression, near to that seen for metastatic melanomas. The expression of fatty acid synthase in congenital nevi could represent either persistence or regression to a fetal immunophenotype, because it is known that fetal cells express high levels of fatty acid synthase (Kusakabe *et al*, 2000). These lesions very rarely occur in the mouth, with no data about FASN expression (Allen and Pellegrini, 1995; Rose *et al*, 2003; Meleti *et al*, 2007). There is no evidence that oral melanocytic nevi increase the risk for oral melanomas, but it is accepted that these melanomas are preceded by pigmented lesions of unknown characteristics (Meleti *et al*, 2007). It would be interesting to determine FASN expression in atypical melanocytic hyperplasias of the mouth, but as they are very rare, a multicentric study would be necessary to select cases of interest.

Our results with cutaneous nevi and melanomas confirm the data of Innocenzi *et al* (2003) and Kapur *et al* (2005), which are similar to the results found in the oral counterparts, i.e., FASN is strongly positive for melanomas and negative for melanocytic nevi. We also confirmed that increased FASN expression is associated with depth of invasion in cutaneous melanomas, and this differed from the results of oral mucosa, where invasive and *in situ* lesions showed similar strong positivity. In cutaneous melanomas, the highest FASN staining intensity was seen in cases with Clark level III and Breslow thickness >2 mm. These data are in accordance with Kapur *et al* (2005) who demonstrated that the immunohistochemical intensity of FASN is associated with Breslow thickness, suggesting this

protein as a molecular prognostic marker. Innocenzi *et al* (2003) showed that patients with cutaneous melanomas, which expressed high levels of FASN, had an increased risk of developing metastases and recurrence of the disease. They also demonstrated that melanomas thicker than 2 mm, with a strong FASN immunoreactivity, were more likely to be lethal than those with comparatively lower expression. Nevertheless, our results suggest that these cutaneous parameters in relation to FASN may not be applied to oral melanomas. In fact, oral melanomas are very aggressive, most with very poor prognosis (Prasad *et al*, 2002).

Considering normal tissues, in the skin, the sebaceous glands are strongly positive for FASN, serving as an internal positive control reflecting the active synthesis of fatty acids. On the other hand, the normal oral epithelium was positive for FASN in the strata granulosum and spinosum and negative in the stratum basale. Those results are consistent with the fact that the keratinocytes of the strata granulosum and spinosum contain about three times more lipids than those of the stratum basale (Uchiyama *et al*, 2000), suggesting that fatty acid synthesis is increased during normal oral epithelium differentiation (Uchiyama *et al*, 2000; Silva *et al*, 2008).

In summary, our findings show that oral melanomas express high levels of fatty acid synthase similarly to their cutaneous counterparts. Nevertheless, FASN staining intensity in cutaneous melanomas increases with increasing depth of invasion, while in oral melanomas, expression seems to be similar. As FASN is practically negative for melanocytic nevi, it can be a useful marker for differential diagnoses of oral and cutaneous benign and malignant melanocytic lesions. Future multicentric studies considering FASN expression in atypical melanocytic lesions of the mouth would be interesting to better understand the biology and development of oral melanomas.

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Author contributions

Adalberto Mosqueda-Taylor, Roman Carlos and Wilson Delgado were responsible to provide the cases of melanomas and nevi for this research. The immunohistochemical reactions were made in the Oral Pathology Laboratory, UNICAMP, coordinated by Professor Oslei Paes de Almeida. Edgard Graner and Jorge Esquiche Leon were responsible for the idea of using antibody FASN in oral melanomas and nevi. The student Bruno Andrade was in charge of writing the article. All authors have read the revised version of this manuscript and they agree with the suggestions made by the reviewers.

References

Allen CM, Pellegrini A (1995). Probable congenital melanocytic nevus of the oral mucosa: case report. *Pediatr Dermatol* **12:** 145–148.

Alli PM, Pinn ML, Jaffee EM, McFadden JM, Kuhajda FP (2005). Fatty acid synthase inhibitors are chemopreventive for mammary cancer in neu-N transgenic mice. *Oncogene* **24:** 39–46.

Baron A, Migita T, Tang D, Loda M (2004). Fatty acid synthase: a metabolic oncogene in prostate cancer? *J Cell Biochem* **91:** 47–53.

Breslow A (1970). Thickness, cross-sectional areas, and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* **172:** 902–908.

Carvalho MA, Zecchin KG, Seguin F *et al* (2008). Fatty acid synthase inhibition with Orlistat promotes apoptosis and reduces cell growth and lymph node metastasis in a mouse melanoma model. *Int J Cancer* **123:** 2557–2565.

Femiano F, Lanza A, Buonaiuto C, Gombos F, Di Spirito F, Cirillo N (2008). Oral malignant melanoma: a review of the literature. *J Oral Pathol Med* **37:** 383–388.

Gansler TS, Hardman W 3rd, Hunt DA, Schaffel S, Hennigar RA (1997). Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. *Hum Pathol* **28:** 686–692.

Heemers H, Maes B, Foufelle F, Heyns W, Verhoeven G, Swinnen JV (2001). Androgens stimulate lipogenic gene expression in prostate cancer cells by activation of the sterol regulatory element-binding protein cleavage activating protein/sterol regulatory element-binding protein pathway. *Mol Endocrinol* **10:** 1817–1828.

Innocenzi D, Alo PL, Balzani A *et al* (2003). Fatty acid synthase expression in melanoma. *J Cutan Pathol* **30:** 23–28.

Jayakumar A, Tai MH, Huang WY *et al* (1995). Human fatty acid synthase: properties and molecular cloning. *Proc Natl Acad Sci* **92:** 8695–8699.

Kapur P, Rakheja D, Roy LC, Hoang MP (2005). Fatty acid synthase expression in cutaneous melanocytic neoplasms. *Mod Pathol* **18:** 1107–1112.

Kridel SJ, Axelrod F, Rozenkrantz N, Smith JW (2004). Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. *Cancer Res* **64:** 2070–2075.

Kuhajda FP (2000). Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition* **16:** 202–208.

Kusakabe T, Maeda M, Hoshi N *et al* (2000). atty acid synthase is expressed mainly in adult hormone-sensitive cells or cells with high lipid metabolism and in proliferating fetal cells. *J Histochem Cytochem* **48:** 613–622.

Kusakabe T, Nashimoto A, Honma K, Suzuki T (2002). Fatty acid synthase is highly expressed in carcinoma, adenoma and in regenerative epithelium and intestinal metaplasia of the stomach. *Histopathology* **40:** 71–79.

Lacasa D, Le Liepvre X, Ferre P, Dugail I (2001). Progesterone stimulates adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression: potential mechanism for the lipogenic effect of progesterone in adipose tissue. *J Biol Chem* **15:** 11512–11516.

Li JN, Gorospe M, Chrest FJ *et al* (2001). Pharmacological inhibition of fatty acid synthase activity produces both cytostatic and cytotoxic effects modulated by p53. *Cancer Res* **61:** 1493–1499.

Meleti M, Mooi WJ, Casparie MK, van der Waal I (2007). Melanocytic nevi of the oral mucosa – no evidence of increased risk for oral malignant melanoma: an analysis of 119 cases. *Oral Oncol* **43:** 976–981.

Prasad ML, Patel S, Hoshaw-Woodard S *et al* (2002). Prognostic factors for malignant melanoma of the squamous mucosa of the head and neck. *Am J Surg Pathol* **26:** 883–892.

Prasad ML, Patel SG, Huvos AG, Shah JP, Busam KJ (2004). Primary mucosal melanoma of the head and neck. A proposal for microstaging localized, stage I (lymphnode-negative) tumors. *Cancer* **100:** 1657–1664.

Rose C, Kaddu S, El-sherif TF, Kerl H (2003). A distinctive type of widespread congenital melanocytic nevus with large nodules. *J Am Acad Dermatol* **49:** 732–735.

Rossi S, Graner E, Febbo P *et al* (2003). Fatty acid synthase expression defines distinct molecular signatures in prostate cancer. *Mol Cancer Res* **1:** 707–715.

Shurbaji MS, Kalbfleisch JH, Thurmond TS (1996). Immunohistochemical detection of a fatty acid synthase (OA-519) as a predictor of progression of prostate cancer. *Hum Pathol* **27:** 917–921.

Silva SD, Agostini M, Nishimoto IN *et al* (2004). Expression of fatty acid synthase, ErbB2 and Ki-67 in head and neck squamous cell carcinoma. A clinicopathological study. *Oral Oncol* **40:** 688–696.

Silva SD, Perez DE, Nishimoto IN *et al* (2008). Fatty acid synthase expression in squamous cell carcinoma of the tongue: clinicopathological findings. *Oral Dis* **14:** 376–382.

da Silva SD, Cunha IW, Nishimoto IN *et al* (2009). Clinicopathological significance of ubiquitin-specific protease 2a (USP2a), fatty acid synthase (FASN), and ErbB2 expression in oral squamous cell carcinomas. *Oral Oncol* **45:** 134–139.

Swinnen JV, Roskams T, Joniau S *et al* (2002). Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. *Int J Cancer* **98:** 19–22.

Takahiro T, Shinichi K, Toshimitsu S (2003). Expression of fatty acid synthase as a prognostic indicator in soft tissue sarcomas. *Clin Cancer Res* **9:** 2204–2212.

Uchiyama N, Yamamoto A, Kameda K, Yamaguchi H, Ito M (2000). The activity of fatty acid synthase of epidermal keratinocytes is regulated in the lower stratum spinousum and the stratum basale by local inflammation rather than by circulating hormones. *J Dermatol Sci* **24:** 134–141.

Visca P, Sebastiani V, Pizer ES *et al* (2003). Immunohistochemical expression and prognostic significance of FAS and GLUT1 in bladder carcinoma. *Anticancer Res* **23:** 335–339.

Weiss L, Hoffmann GE, Schreiber R *et al* (1986). Fatty-acid biosynthesis in man, a pathway of minor importance. Purification, optimal assay conditions, and organ distribution of fatty-acid synthase. *Biol Chem Hoppe Seyler* **367:** 905–912.

Zhou W, Simpson PJ, McFadden JM *et al* (2003). Fatty acid synthase inhibition triggers apoptosis during S phase in human cancer cells. *Cancer Res* **63:** 7330–7337.