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Report

# *SLURP-1* is mutated in Mal de Meleda, a potential molecular signature for melanoma and a putative squamous lineage tumor suppressor gene

**Christina Bergqvist[1,*], MD, Humam Kadara[2,*], MD, Lamiaa Hamie[3], MD, Georges Nemer[2], MD, Remi Safi[1], MD, Mirna Karouni[1], MD, Nadine Marrouche[4], MD, Ossama Abbas[1], MD, Divina J. Hasbani[5], MS, Abdul G. Kibbi[1], MD, Dany Nassar[1,†], MD, Yutaka Shimomura[6,†], MD, and Mazen Kurban[1,2,7,†], MD**

[1]Department of Dermatology, American University of Beirut, [2]Department of Biochemistry and Molecular Genetics, American University of Beirut, [3]Department of Internal Medicine, American University of Beirut, Beirut, Lebanon, [4]Department of Dermatology, Norfolk and Norwich University, Norwich, UK, [5]Medical Student, American University of Beirut, Beirut, Lebanon, [6]Division of Dermatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, and [7]Department of Dermatology, Columbia University, New York, NY, USA

**Correspondence**
Mazen Kurban, MD
Department of Dermatology and Biochemistry and Molecular Genetics
American University of Beirut Medical Center
Beirut
Lebanon
Department of Dermatology
Columbia University Medical Center
New York, NY, USA
E-mail: mk104@aub.edu.lb

*Equally contributing co-first authors.
†Equally contributing co-corresponding authors.

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## Abstract

**Background** Mal de Meleda (MDM) is a rare inherited autosomal recessive genodermatosis characterized by palmoplantar keratoderma (PPK) with transgrediens and caused by mutations in the *SLURP1* gene. Uncommonly, cutaneous tumors have been found at PPK sites in MDM patients.

**Objective** To study a Middle Eastern family with MDM with both PPK and skin tumors.

**Methods** We studied a Middle Eastern (Palestinian) family with clinical features of MDM and cutaneous tumors. Histopathological analysis was performed on biopsies from skin lesions found in the affected individuals. Direct sequencing of *SLURP1* was performed in MDM affected members. *In silico* analysis of publicly available datasets was used to survey *SLURP1* mRNA levels in normal and malignant tissues. Statistical analysis was performed in the R statistical language.

**Results** Affected members from the Middle Eastern family displayed severe forms of PPK consistent with MDM. Histopathological analysis of the skin lesions revealed that the examined affected members exhibited skin squamous cell carcinomas (SCCs) and melanoma. Sequence analysis revealed homozygous *SLURP1* mutations (c.82delT) in the affected members. Following analysis of various publicly available expression datasets, *SLURP1* mRNA levels were found to be markedly elevated in tissues of epithelial lineage, relative to tissues of other lineages, and significantly suppressed in malignant tumors of epithelial lineage relative to normal or their premalignant counterparts. There was significant decrease in *SLURP-1* expression in melanomas versus melanocytic nevi as well as a highly significant decrease in *SLURP-1* expression in metastatic melanomas as compared to primary melanoma.

**Conclusion** Our study underscores cases of Middle Eastern MDM with *SLURP1* mutations and skin malignancies at PPK sites. Our findings also highlight a plausible epithelial lineage-specific tumor suppressor role for the SLURP1 gene, as well as a role in the development and metastasis of melanoma and thus a potential molecular signature for melanoma.

## Introduction

Palmoplantar keratodermas are a heterogeneous group of acquired or inherited disorders characterized by thickening of the palms and soles.[1] Mal de Meleda (MDM), a type of palmoplantar keratoderma (PPK), is a rare autosomal recessive disorder first described by Luca Stulli in 1826 on the Dalmatian island of Meleda (now Mljet).[2] This disorder is characterized by early onset, soon after birth, of thick and diffuse hyperkeratosis of the palms and soles, with prominent erythematous borders with transgrediens (lesions spreading to the dorsal surfaces of hands and feet) and progradiens (progressing with age) features. The keratoderma can be severe enough to cause disfigurement of hands and feet leading to severely impaired function.[1,3]



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Earlier reports have shown that MDM occurs secondary to mutations in the *SLURP1* gene (previously ARS-B) encoding a secreted toxin-like mammalian lymphocyte antigen 6/urokinase-type plasminogen activator receptor-related protein 1 (SLURP1) found in epithelium, sensory neurons, and immune cells.[4–6] SLURP1 protein is a paracrine/autocrine hormone and an allosteric agonist to the alpha 7 nicotinic acetylcholine receptor (nAChR).[6] The protein was shown to play roles in transmembrane signal transduction,[7] cell adhesion and cell activation,[8] regulation of epidermal homeostasis,[5] and normal T-cell activation,[5] and to mediate inflammation and malignant cell transformation.[6] The mechanism of the antiproliferative activity of SLURP1 has recently been probed and was suggested to be linked to α7-nAChR signaling.[6] Interestingly, alpha 7 nAChR plays a central role in differentiation of the stratified squamous epithelium; keratinocyte alpha 7 nAChR regulates homeostasis and terminal differentiation of epidermal keratinocytes required for formation of the skin barrier.[9]

In this report, we studied a Middle Eastern (Palestinian) family with MDM that presented with both severe PPK and skin lesions. We found that two members of this family developed cutaneous overgrowth within the hyperkeratotic area. Histopathological analysis was then performed on biopsies from these lesions. Direct sequencing of *SLURP1* was performed in MDM-affected members. Next, *in silico* analysis of publicly available datasets was used to survey *SLURP1* mRNA levels in normal and malignant tissues of all lineages in an attempt to investigate a possible role for SLURP1 in tumor development.

## Materials and Methods

### Patients

Two members of a Palestinian family were presented to the Department of Dermatology at the American University of Beirut Medical Center. The family pedigree is described in Figure 1a. One of the affected individuals, II-6, was a 46-year-old woman who presented in 2011 with progressively worsening severe erythematous exfoliating PPK which developed since early infancy (Fig. 1b,d). We also studied a second affected member, II-10, who was a 42-year-old man with similar PPK.

### Skin biopsies and histological examination

A skin biopsy was obtained from the cutaneous mass at the base of the left thumb of patient II-6, as well as from both masses located over the dorsum of the left wrist of patient II-10. The biopsies were formalin-fixed and paraffin-embedded (FFPE). Slides were prepared from 4 μ sections of the FFPE blocks and were stained with hematoxylin and eosin. Histopathological examination was independently performed by two dermatopathologists (OA and AGK).

### Sequence analysis

After obtaining written informed consents, we collected peripheral blood samples from the family members and unrelated healthy control individuals in EDTA-containing tubes.

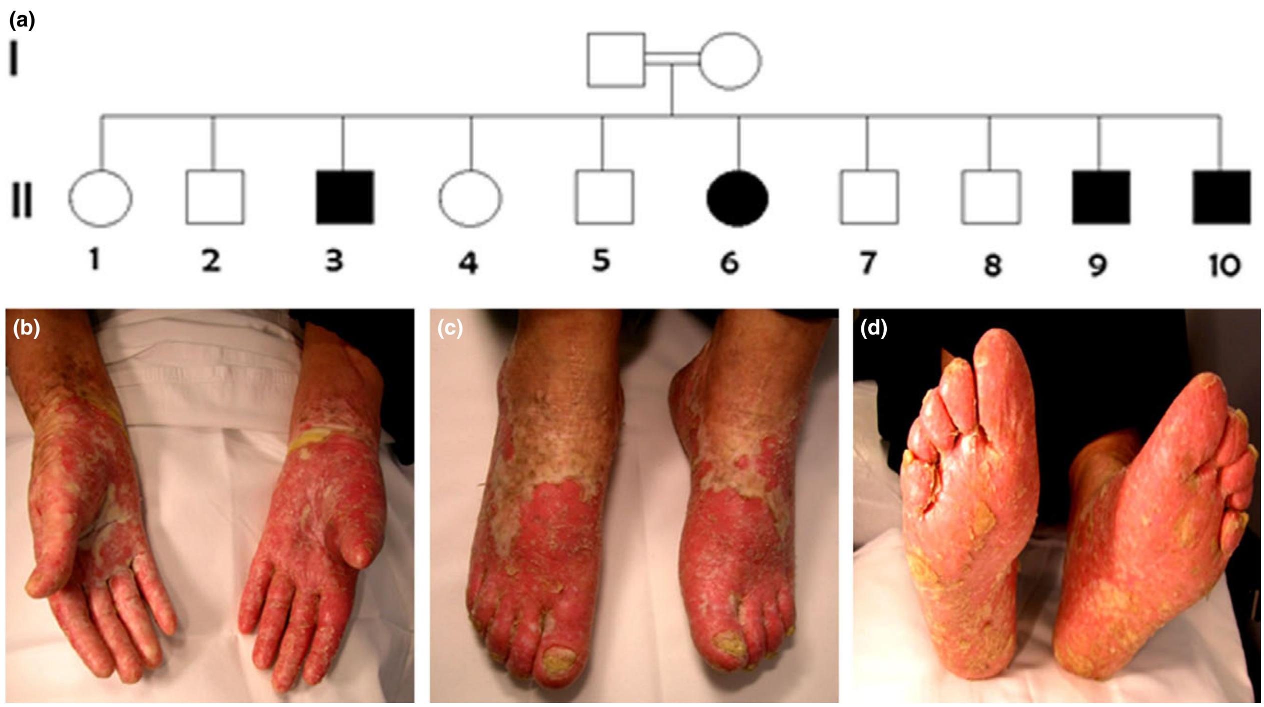


**Figure 1** A consanguineous Middle Eastern family with Mal de Meleda (MDM). (a) A consanguineous Palestinian family with four MDM-affected members. Member II-6 was a 46-year-old woman who was found to have progressively worsening severe erythematous exfoliating palmoplantar keratoderma with transgrediens (b–d)



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Genomic DNA was isolated from peripheral blood lymphocytes according to standard extraction techniques. The study was approved by the Ethics Committee of the American University of Beirut Medical Center and Niigata University Medical Center and in adherence to the declaration of Helsinki Principles. All exons and exon-intron boundaries in *SLURP1* were amplified as previously described.[10] The amplified PCR products were directly sequenced using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, USA) and the ABI 3100 Genetic Analyzer. Sequence results were analyzed using the Applied Biosystems software and compared by alignment to sequence in the UCSC browser.

### *In silico* analysis of *SLURP1* expression

Expression levels of *SLURP1* mRNA were assessed in an array set comprised of pan-normal samples (GSE7307 from the gene expression omnibus). Statistical analysis of the correlation of *SLURP1* expression with various squamous markers in normal tissues was performed using the Pearson's correlation method. *SLURP1* expression was analyzed in a publicly available dataset by Tantalov and colleagues comprised of 18 benign nevi and 45 melanomas[11] and in a set of primary ($n = 31$) and metastatic ($n = 52$) melanomas.[12] *SLURP1* expression was also compared and contrasted between tumors and normal tissues in publicly available microarray datasets consisting of: cervical squamous cell carcinomas by Scotto *et al.* (21 normal and 32 tumors[13]), and Zhai and colleagues (10 normal and 21 tumors[14]), squamous esophageal cancers (17 tumor and normal pairs[15]), and head and neck squamous cell carcinomas (13 normal and 41 tumors[16]). *SLURP1* expression was also assessed in a dataset that included the histopathological sequence of esophageal adenocarcinoma (28 normal esophageal tissues, 15 preneoplastic esophageal tissues from patients with Barrett's esophagus, and 75 tumors[17]). Expression of *SLURP1* mRNA was statistically compared and contrasted between the different samples using *t*-tests. Statistical analyses, including correlation of *SLURP1* with squamous markers and graphical displays, were performed in the R language and environment.

## Results

### Identification of cutaneous malignancies in a Middle Eastern family with MDM

We studied a Middle Eastern (Palestinian) family (Fig. 1a) with two members who were diagnosed with MDM based on clinical presentation. Member II-6 was a 46-year-old woman who was found to have progressively worsening severe erythematous exfoliating PPK which had developed in the patient since early infancy (Fig. 1b,d). Upon further examination, this patient had an ulcerated skin lesion at the base of the left thumb suspicious of a skin cancer (Fig. 2a). The other member with MDM and similar PPK, II-10, also harbored skin lesions over the dorsum of the left wrist (Fig. 2b,c). These two lesions consisted of a large multilobulated black ulcerating nodule that had been growing over 4 years and an erythematous hyperkeratotic ulcerated nodule estimated to have been progressing over a 4-month period (Fig. 2b,c). Histopathological examination revealed that the ulcerating lesion of member II-6 was an invasive and moderately differentiated SCC (Fig. 3a,b). The black nodule of member II-10 was consistent with invasive melanoma (Fig. 3c, d), and that of the ulcerated hyperkeratotic nodule showed infiltrating and keratinizing SCC (Fig. 3e). Survey of the literature for similar observations identified few studies reporting cutaneous lesions and melanoma arising in the hyperkeratotic lesions of MDM patients, which are reviewed in Table 1.

### Identification of *SLURP1* mutations in Middle Eastern MDM patients

We collected peripheral blood cells from the affected individuals and subsequently performed genomic DNA isolation from the cells; all exons and exon-intron boundaries for *SLURP1* were

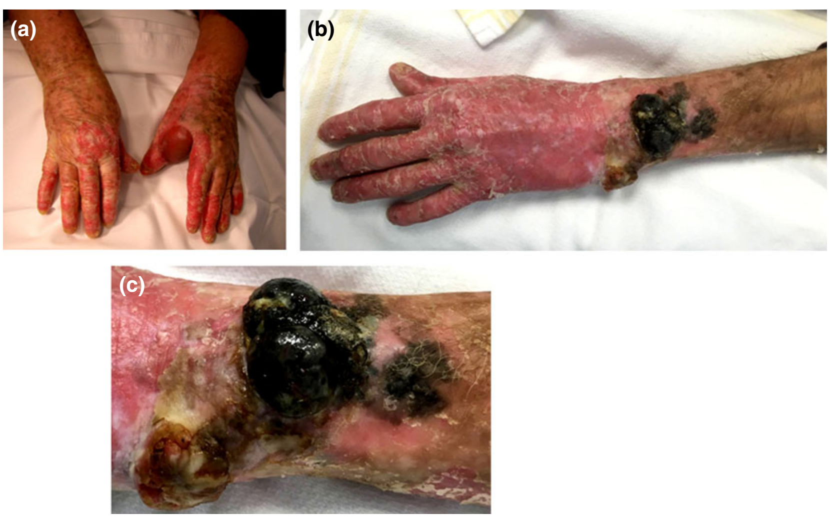


**Figure 2** Clinical observation of cutaneous tumors in Middle Eastern MDM patients. (a) Affected member II-6 exhibited an ulceration at the base of the left thumb (no picture available) that was suspicious for skin malignancy and subsequently operated upon and covered using a graft (depicted in the Figure). Affected member II-10 showing two adjacent skin nodule lesions over the dorsum of the left wrist, a black ulcerating nodule (b), and an erythematous hyperkeratotic ulcerated nodule (c)

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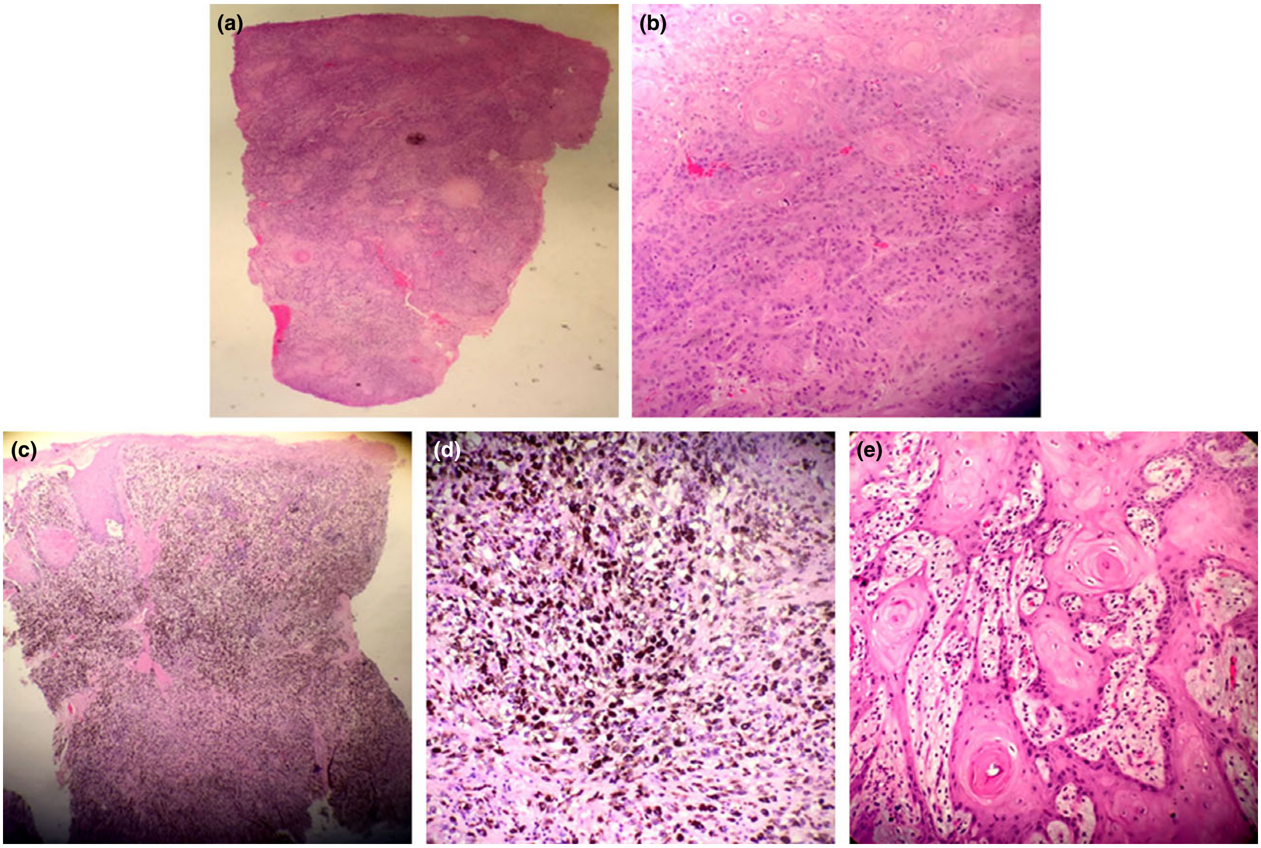


**Figure 3** Histopathological examination of the skin lesions in the Middle Eastern MDM patients. Histopathological analysis of the observed skin lesions was performed following H&E staining and as described in the Materials and Methods section. The ulcerated lesion from affected member II-6 was found to be consistent with invasive moderately differentiated squamous cell carcinomas (a, b) (a – Hematoxylin and eosin stain; original magnification ×4, b – Hematoxylin and eosin stain; original magnification ×10). The black ulcerating nodule from individual II-10 was found to be consistent with invasive malignant melanoma, nodular subtype (c, d) (c – Hematoxylin and eosin stain; original magnification ×4, d – Hematoxylin and eosin stain; original magnification ×20). (e) The cauliflower-like erythematous hyperkeratotic ulcerated nodule from patient II-10 was consistent with invasive keratinizing SCC (Hematoxylin and eosin stain; original magnification ×40)

**Table 1** Malignant melanomas associated with the palmoplantar keratodermas of Mal de Meleda patients

| Authors | Melanoma location | Year | Reference |
|---|---|---|---|
| Shimizu *et al.* | Right 4th finger | 1959 | 37 |
| Matsuda *et al.* | Left fingers | 1979 | 38 |
| Okada *et al.* | Right sole | 1979 | 39 |
| Arisu *et al.* | Left sole | 1991 | 40 |
| Aygit *et al.* | Left little finger | 1999 | 41 |
| Mozzillo *et al.* | Right-hand 3rd, 4th, and 5th fingers | 2003 | 30 |
| Sartore *et al.* | Dorsal surface of left hand | 2009 | 31 |
| Tjiu *et al.* | Right inner ankle | 2010 | 5 |
| Vilas-Sueiro *et al.* | Right ankle | 2016 | 32 |

amplified, and subsequently the PCR products were directly sequenced. Notably, all affected individuals were homozygous for a deletion mutation at position 82, designated c.82delT. The mutation was not found in 200 control chromosomes from the same population (Fig. 4). In accordance with reports on MDM families in different regions,[4,18–20] these findings further point to the probable pathogenic role of *SLURP1* deleterious mutations in the pathogenesis of Middle Eastern MDM.

### Expression of *SLURP1* normal and neoplastic tissues

Our findings on the presence of cutaneous malignancies in MDM patients with *SLURP1* mutations prompted us to begin to explore the association of *SLURP1* with skin malignancies by assessing expression levels of the gene in normal and malignant tissues. We first probed the expression of *SLURP1* in a publicly available dataset (GSE7307 from the gene expression omnibus, see Materials and Methods) comprised of pan-normal tissues from various organs. This analysis demonstrated that *SLURP1* expression was highly expressed in normal tissues of squamous lineage (e.g., cervix, esophagus, skin, and head and neck) relative to other normal epithelia and specimens (Fig. 5;

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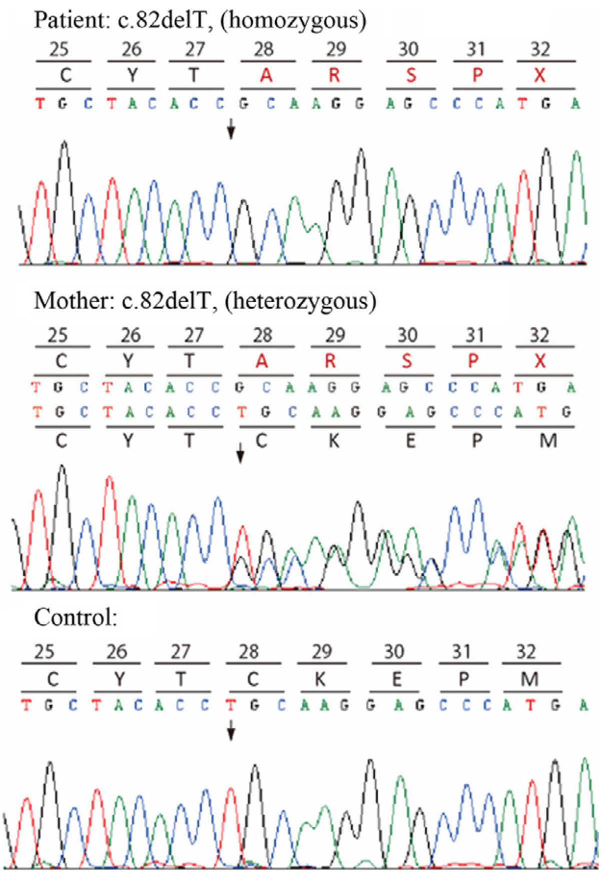


**Figure 4** Identification of germline mutation in the *SLURP1* gene in Middle Eastern MDM. Genomic DNA was isolated from acquired blood cells from the affected members, and all exons of *SLURP1* were sequenced as described in the Materials and Methods section. Direct sequence analysis from the family revealed a homozygous deletion mutation designated c.82delT as well as heterozygous mutation found in carriers. The mutation was not detected in 200 control chromosomes from the same population

all $P < 10^{-8}$). Of note, we also found that *SLURP1* expression was markedly and significantly correlated with expression of various squamous markers including keratins and transglutaminase 1 (*TGM1*) (all $P < 0.001$; Fig. S1). We next statistically compared and contrasted *SLURP1* expression in normal tissues and lesions (preneoplastic or neoplastic) using publicly available microarray datasets (see Materials and Methods). We found that *SLURP1* expression was significantly decreased in cutaneous melanoma compared to preneoplastic skin lesions (benign nevi) (Fig. 6a, left; $P = 1.8 \times 10^{-6}$) and in metastatic compared with primary melanocytic lesions (Fig. 6b, right; $P = 1.1 \times 10^{-8}$) pointing to the probable implication of *SLURP1* in onset and progression of melanoma. We also found that *SLURP1* expression was markedly and significantly decreased in several squamous tumors compared to their normal counterparts including cervical SCC (Fig. 6c,d; both $P < 0.0001$), esophageal SCC (Fig. 6e, left; $P = 1.1 \times 10^{-11}$), and head and neck SCC (Fig. 6f right; $P < 10^{-15}$). Of note, we also found, by statistical analysis of a publicly available dataset by Kim and colleagues,[17] that *SLURP1* was significantly decreased in both Barrett's esophagus and esophageal adenocarcinoma relative to normal esophagus (Fig. S2, both $P < 0.0001$) pointing to aberrant *SLURP1* patterns, the histopathological sequence of esophageal adenocarcinoma. These findings strongly suggest that downregulation of *SLURP1* expression may be implicated in the pathogenesis of various solid tumors, particularly malignancies of squamous lineage, and thus this gene may be a squamous lineage-specific tumor suppressor.

## Discussion

Genodermatoses comprise rare inherited skin disorders commonly encountered in regions with high rates of consanguinity. Currently, there are no available treatments for genodermatoses, such as MDM, causing patients to abandon long-term clinical follow-up, which result in missing some features of the condition that present late in the disease. Our group among others has had a long standing interest in understanding the molecular pathogenesis of genodermatoses in addition to unraveling uncommon clinical features of these diseases.[21,22] In this report, we sought to study a consanguineous Middle Eastern family with clinical features of MDM, a rare autosomal recessive genodermatoses. Notably and upon further clinical evaluation and follow-up, two members of this family were found to develop cutaneous malignancies, SCCs, and melanoma, all developing at the sites of the keratoderma. We found that these observations were in accordance with a very limited number of studies performed in different regions (Table 1). Genetic analysis revealed that the patients displayed germline pathogenic mutation in *SLURP1* (c.82delT mutation), emphasizing again the implication of this gene in the pathogenesis of Middle Eastern MDM. Of note, we found in our Middle Eastern MDM patients the same variant of *SLURP1* previously reported in the study by Fischer and colleagues.[4] To date, the c.82delT mutation is the most common cause of MDM in the world which points toward a hot spot for mutations.[18] The c.82delT mutation has been reported to be the most common mutation in the Mediterranean population and in regions with high rates of consanguinity.[19]

Lastly, we found that *SLURP1* expression was specific to normal tissues of squamous lineage (e.g., skin, cervix, head, and neck) and was markedly suppressed in squamous tumors relative to normal tissues rendering the plausible supposition that this gene may function as a squamous lineage-specific tumor suppressor.

Earlier studies have interrogated molecular aberrations involved in the pathogenesis of MDM. These studies demonstrated that the mammalian lymphocyte antigen 6/urokinase-type plasminogen activator receptor-related protein 1 (*SLURP1*) was mutated in MDM patients from Croatia and North Africa (Algeria).[4] Our study shed light on the presence of mutations in

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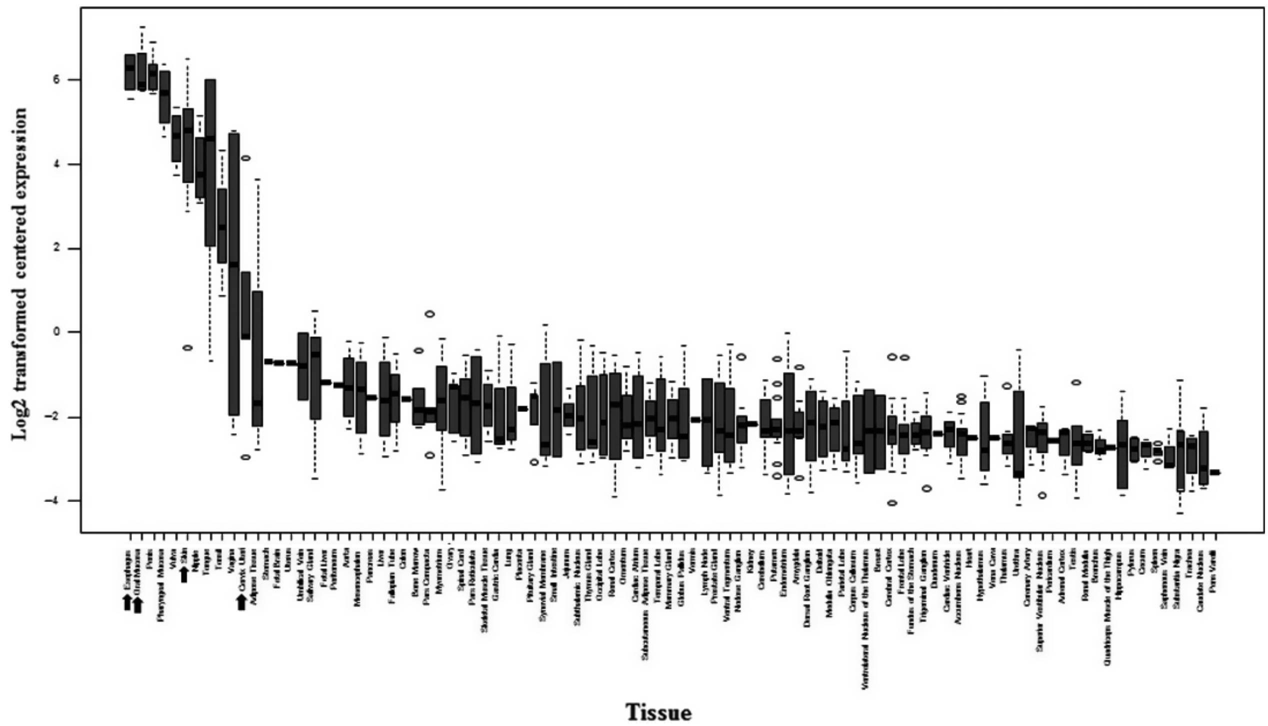


**Figure 5** Increased *SLURP1* expression in normal tissues of squamous lineage. Expression levels of *SLURP1* mRNA were assessed in an array set comprised of pan-normal samples (GSE7307 from the gene expression omnibus) as described in the Materials and Methods section. Log (base 2) median-centered expression values for *SLURP1* were plotted. Boxes represent 25–75% expression ranges, and whiskers constitute maxima and minima. Solid horizontal lines represent median *SLURP1* mRNA log (base 2) median-centered expression values

the *SLURP1* gene in Middle Eastern MDM patients. Of note, we found in Middle Eastern MDM patients the same variant of *SLURP1* previously reported in the study by Fischer and colleagues.[4] Eckl *et al.* reported a large consanguineous Palestinian pedigree where patients were homozygous for a new mutation that substitutes an arginine for a conserved glycine residue at position 86.[20] These findings suggest that *SLURP1* mutations are also pathogenic in Middle Eastern MDM.

In this report, we noted cutaneous SCCs and melanoma in Middle Eastern MDM patients. It is worthwhile to mention that a limited number of hereditary PPKs have been associated with skin malignancies (cutaneous melanomas or SCCs) within the hyperkeratotic lesions.[23–27] Squamous cell carcinomas have been associated with the PPK in patients with Olmsted syndrome[27] and Huriez syndrome.[28] To the best of our knowledge, this is the third case of squamous cell carcinoma to be reported in patients with MDM as previously described were a case of Bowen's disease (SCC *in situ*)[29] and one case of ungual cutaneous SCC.[25] Several cases of melanoma arising in the hyperkeratotic lesions of MDM, patients have been previously reported (summarized in Table 1).[5,30–33] In addition, a previous study demonstrated that Papillion-Lefèvre syndrome was associated with cutaneous melanoma.[23] It is noteworthy that reports interrogating MDM in Middle Eastern patients are extremely limited. A previous study revealed *SLURP1* mutations in Middle Eastern MDM patients from Palestine and the United Arab Emirates, yet no skin tumors were described in those patients.[20] Our findings are in corroboration with few previous studies but now extending the observation of skin malignancies in MDM to Middle Eastern patients.

Prompted by the findings of both *SLURP1* mutations in Middle Eastern MDM patients and the incidence of skin malignancies in those patients, we surmised that aberrant *SLURP1* expression may be implicated in the pathogenesis of malignancies of squamous epithelial lineage, as well as melanoma. We surveyed various publicly available expression datasets of pan-normal tissues as well as sets comprised of various squamous malignant lesions and normal tissues. These *in silico* analyses revealed two main expression patterns for *SLURP1*: (1) markedly elevated expression in normal squamous tissues such as skin, esophagus, head and neck, and cervix relative to normal tissues from other organs and (2) suppression in squamous malignant lesions (e.g., squamous cell carcinoma) relative to normal squamous tissues. Interestingly, *SLURP1* expression was markedly decreased in melanoma as compared to melanocytic nevi. This decrease was more pronounced in metastatic versus primary melanoma. These data point to probably tumor suppressor roles for *SLURP1* in squamous malignancies such



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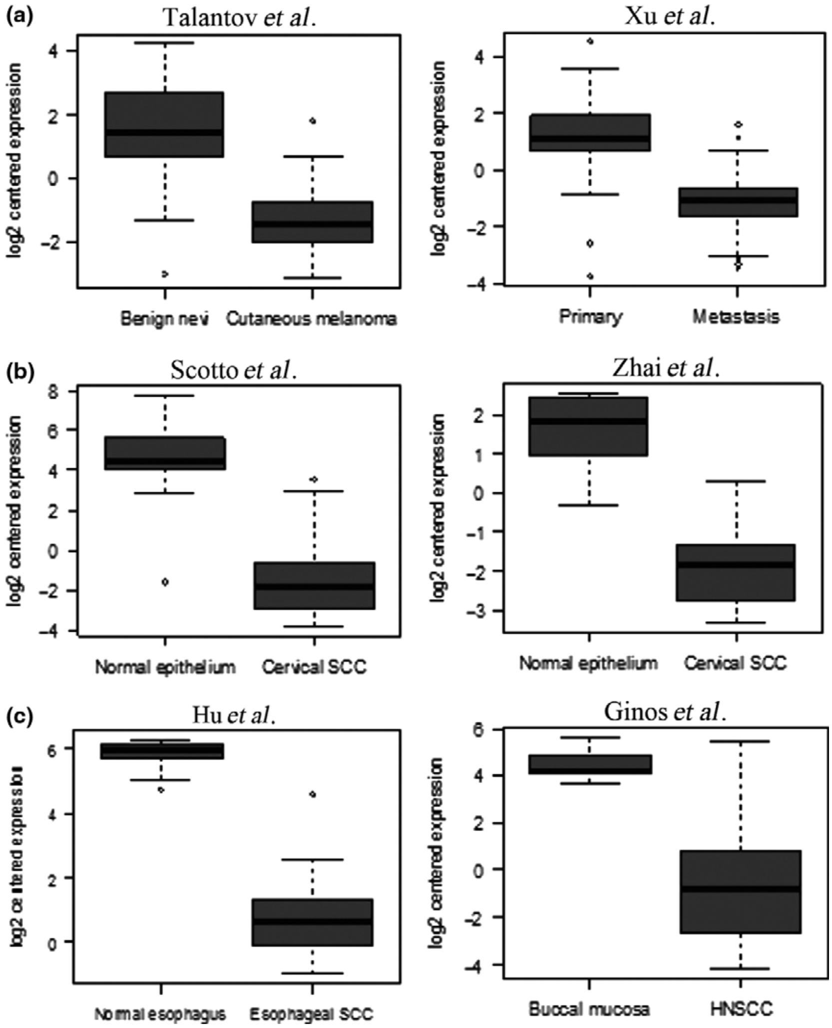


**Figure 6** Decreased *SLURP1* expression in squamous premalignant and malignant lesions. Expression of *SLURP1* mRNA was statistically analyzed in datasets comprised of benign nevi and cutaneous melanoma (a, left panel[11]), primary and metastatic melanomas (a, right panel[12]), cervical SCCs and normal tissues (b[13,14]), esophageal SCCs and normal tissues (c, right panel[15]), and head and neck squamous cell carcinomas (HNSCCs) and normal buccal mucosa (c, right[16]). Log (base 2) median-centered expression values for *SLURP1* were plotted. Statistical significance of differences in *SLURP1* mRNA levels was assessed using *t*-tests. Boxes represent 25–75% expression ranges, and whiskers constitute maxima and minima. Solid horizontal lines represent median *SLURP1* mRNA expression values

as of the skin, as well as in melanoma. It is also intriguing to speculate that *SLURP1* may be harnessed as a potential molecular signature for progression of nevi to primary melanomas or in the progression of primary melanomas to metastatic lesions, a supposition that warrants further studies. It is worthwhile to note that *SLURP1* is highly involved in skin homeostasis as well as the immune system.[34] It has been previously postulated that the increased incidence of malignant melanoma in MDM patients is, in part, because of inactivation of proapoptotic signaling downstream of *SLURP1* as well as deficient T-cell activation, immune surveillance, and constant inflammation in the hyperkeratotic skin.[5] This study, using *in vitro* analyses on blood samples, showed that transfection of T-lymphocytes with wild-type *SLURP1* restored T-cell activation in MDM patients.[5] Moreover, *SLURP1* was shown to be an allosteric agonist of the nicotinic acetylcholine receptors, which in turn were demonstrated in murine models to be essential for the activation of T-lymphocytes.[5] Furthermore, *SLURP1* was shown to exhibit proapoptotic roles to control keratinocyte regulation and turnover.[35] It is noteworthy that alpha 7 nAChR plays a central role in the differentiation of the stratified squamous epithelium[9] and that aberrations in the nicotinic acetylcholine receptor pathway have been implicated in other malignancies such as those of the lung, stomach, and pancreas,[36] with the implication of genes with similar roles to *SLURP1*. Based on our expression analyses, along with the aforementioned previous studies probing the intracellular role of *SLURP1*, it is plausible that *SLURP1* may function as a squamous lineage tumor suppressor gene in various malignancies (of squamous lineages) as well as an important role in melanoma formation and metastasis.

In conclusion, we studied Middle Eastern MDM cases and found that those patients exhibited typical clinical features of this rare genetic skin disorder along with an incidence of overt skin SCCs and melanoma. We also found that those Middle Eastern MDM patients exhibited mutations in the *SLURP1* gene that are pathogenic. Lastly, by surveying various publicly available datasets, we found that SLURP1 is preferentially

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expressed in squamous normal tissues and is markedly suppressed in various squamous premalignant and malignant lesions, as well as melanocytic nevi and melanoma, respectively, relative to normal tissues. Our study demonstrates that *SLURP1* may be involved in the pathogenesis of Middle Eastern MDM and also function as a squamous lineage-specific tumor suppressor gene and melanocytic-derived tumors.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Correlation of *SLURP1* and mRNA levels with expression of squamous markers in normal tissues.

**Figure S2** Decreased *SLURP1* expression in lesions comprising histopathological sequence of esophageal adenocarcinoma development.

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