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Estrogen receptor β expression in nevi during pregnancy

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Abstract

Estrogen levels increase during pregnancy and clinical evidence has long suggested that melanocytes are estrogen-responsive. We hypothesized that nevi from pregnant patients would exhibit increased expression of estrogen receptor β (ER β) and thus enhanced potential to respond to altered estrogen levels. Normal, dysplastic and congenital nevi ($n = 212$) were collected from pregnant and non-pregnant women ranging from 18 to 45 years of age. Immunohistochemical staining was performed on these nevi using antibodies specifically directed against estrogen receptor α (ER α) and ER β . ER α was not observed in any lesions; thus, ER β was the predominant estrogen receptor in melanocytic cells from all types of nevi. Enhanced positivity for ER β in normal nevi during pregnancy was noted, compared with non-pregnant controls including nevocytes residing in both the epidermal and dermal micro-environments ($P = 0.005$ and $P = 0.001$ respectively). Nevi with increasingly melanocytic atypia showed increased ER β in nevocytes nested within the epidermis. No additional increase in ER β in atypical nevi was observed during pregnancy. For normal and congenital nevi, regardless of pregnancy status, dermally associated nevocytes tended to have greater ER β immunoreactivity. Significant decreases in ER β immunoreactivity were observed in congenital nevi from pregnant women compared with normal and dysplastic nevi from pregnant women. Our data suggest that nevi possess the capacity to be estrogen-responsive. Factors such as pregnancy and degree of atypia are associated with enhanced ER β with the exception of congenital nevi where the melanocytes were unique in their response to pregnancy.

Keywords

estrogen receptor β ; immunohistochemistry; nevi; nevocytes; pregnancy

Introduction

The impact of pregnancy on nevi and malignant melanoma has led to much debate. Pregnancy has long been associated with clinical observations of skin hyperpigmentation along with nevus darkening followed by subsequent regression after parturition (1-5). However, many of these reports have been criticized because of their reliance on subjective reports from patients regarding nevus size and colour changes (6). In general, histologic comparison of nevus samples from pregnant women to non-pregnant controls have failed to note a significant

difference in cytologic atypia between the study and the control groups (4,6-8). However, one series did report a trend towards increased histopathological atypia in nevi from pregnant patients (8). A photographic, clinical and histological analysis of patients with the dysplastic nevus syndrome (DNS) (9) used clinical photographs and histopathology to document both a macroscopic increase in nevi changes and histopathological dysplasia during pregnancy. Similar observations in pregnant patients without DNS were not demonstrated (5,10). These data suggest that nevi from patients with DNS may have a greater propensity to change during pregnancy compared with nevi from normal patients.

New theories and approaches have been advanced to explain the interplay between hormones and changes in nevi. One proposal is that alterations in single, isolated melanocytes may be solely responsible for the nevocytic changes during pregnancy rather than nests of melanocytes in nevi. Such a focal occurrence theory would explain why macroscopic changes may not always be evident in nevi during pregnancy (10). The use of traditional dermoscopy, as well as the recent advent of computerized (digital) dermoscopic analysis, has allowed for more objective and detailed evaluation of nevi during pregnancy. The availability of this new imaging modality has resulted in the observation of significant changes including increased vascularity, disorganized architecture and increased pigmentation (with darker globules and thicker pigment network on diascopy) in nevi during pregnancy (5,11-13). Nevertheless, the present consequence of hormonal fluxes during pregnancy with regard to melanocytic response remains speculative.

The current uncertainties surrounding nevi and pregnancy find their roots in the early 1950s, when case reports and studies linked pregnancy to an increased risk of malignant transformation of nevi as well as a poorer prognosis for women diagnosed with melanoma during pregnancy (14). Subsequent epidemiologic studies controlling for tumor size and stage reported no difference in survival rates between pregnant and non-pregnant women with melanoma (15-19). However, an increased tumor thickness has been noted in pregnant patients in a couple of studies, although this was not always statistically significant (15,20). Further questions were raised by a 2007 case-control study of 318 women, which found that pregnancy, increased numbers of pregnancies and changes in nevi during recent pregnancies were associated with an increased incidence of melanoma before the age of 55 (21).

Despite strong clinical impressions of a relationship between pregnancy and melanocytic responsiveness, no reports have successfully established a linkage between hormone responsiveness and nevi during pregnancy. The absence of estrogen receptor α (ER α) in melanocytes and melanoma discouraged further investigation (22). Some years later, a second form of the receptor, estrogen receptor β (ER β) was discovered (23), and found to be an important component of estrogen signalling (24). Our group has reported that ER β , and not ER α , is the prevailing estrogen receptor in normal nevi, dysplastic nevi and malignant melanomas (25). Furthermore, we noted that ER β expression is highest in dysplastic nevi and lentigo maligna, but shows a measurable decrease as malignant melanoma cells are found at progressively deeper levels in the dermis (25). The exact role of ER β in melanocytes remains unknown and the contradictory results in the literature indicate the need for additional work on the role of estrogens in melanocytic functions (26). Given the high incidence of melanoma in women of child-bearing age and the need to manage women clinically during pregnancy to prevent melanoma (27,28), further investigation into the hormonally responsive nature of these cells, as well as the potential for hormonally driven malignant transformation, is warranted. The goal of the present study was to examine the potential for estrogen responsiveness of nevocytes during pregnancy. This was accomplished by comparing levels of ER α and ER β immunoreactivity, the two estrogen receptors believed to be responsible for the cellular response to estrogen, in normal, dysplastic and congenital nevi in pregnancy with age-matched non-pregnant controls.

Materials and methods

Tissue acquisition

Nevi from pregnant and non-pregnant women between the ages of 18–45 years old were collected in the Dermatology and Plastic Surgery clinics with approval from the Vanderbilt University Institutional Review Board. Patients were consented and informed of the study requirements and all patient information was kept confidential in accordance with HIPPA regulations. Additional archived nevi were provided by the Tissue Acquisition Core Lab with support from the Cancer Center Support Grant (P30 CA68485-08). Nevi were subsequently divided into two primary groups depending on whether the patient was pregnant ($n = 23$) or non-pregnant ($n = 100$) at the time of biopsy. Upon clinical evaluation and dermatopathological confirmation by biopsy, the nevi were secondarily categorized into three sub-groups: normal, dysplastic and congenital (Table 1). In the normal nevus, non-pregnant category, we noted that the data from 11 of 82 lesions were previously utilized whereas in the dysplastic nevus, non-pregnant category the data from 23 of 62 lesions were utilized in data analysis in an earlier report (25). These data were used to augment the total numbers of nevi in each category.

Immunohistochemical staining

Immunohistochemical staining was performed using commercial antibodies specifically directed against ER α and ER β . The immunohistochemical protocol for this study was identical to a recent study at our institution exploring ER β expression and distribution within a variety of melanocytic lesions (25). Formalin-fixed paraffin-embedded nevi were sectioned at 5 μ m thickness, placed on slides and warmed overnight at 50°C to adhere sections to the glass slides. The slides were then deparaffinized and rehydrated with graded alcohols ending with a Tris-buffered saline wash (TBS-T Wash Buffer; Dako, Carpinteria, CA, USA). Antigen retrieval was performed in a Decloaker apparatus (Biocare Medical, Walnut Creek, CA, USA) with a citrate buffer at pH 6.0 (Dako) using the following protocol: SP1 125°C for 4 min, SP2 90°C for 10 s, SP-Limit 10°C. Once completed, slides were removed from the Decloaker, cooled for 20 min and washed with TBS-T. Slides were then bleached overnight with 3% H₂O₂ to remove excess of melanin pigment that might obscure the chromogen. Non-specific background staining was minimized by washing slides in TBS followed by a 20-min incubation in serum-free protein block (Dako). Primary antibody to either ER α (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or polyclonal ER β (Upstate, New York, NY, USA) was applied to the slides at a dilution of 1:250 and incubated for 2 h. This was followed by incubation with a biotinylated secondary antisera and streptavidin HRP (LSAB + kit, Dako). Slides were rinsed with TBS-T between each reagent treatment and finally visualization was achieved with the application of Nova Red (Vector Laboratories, Burlingame, CA, USA) for 10 min at room temperature. All slides were counter-stained with Mayer's haematoxylin for 30 s, dehydrated through a series of alcohols and xylenes, and then cover-slipped with Acrytol Mounting Media (Surgipath, Richmond, IL, USA). Microscopic examination and imaging of slides were performed using an Olympus BH-2 microscope with a Polaroid digital microscope camera 2 (Olympus, Melville, NY, USA).

Grading of nevi responsiveness

Evaluation and grading of the immunoreactivity were performed by three separate individuals in a blinded fashion by scoring percentage of nevocytes that exhibited nuclear staining (Table 2). When lesions were large and were therefore divided and distributed as multiple sections within a paraffin block, all tissue sections from this single specimen were examined and a final, single scoring determination was made. When discordance was occasionally noted among scorings on a given sample, three investigators re-evaluated this situation, discussed the discrepancies and arrived at a mutual concordance. Although ER β is a nuclear receptor and nuclear immunoreactivity was the norm, unusual variations were also noted in the cytoplasmic

distribution of ER β so that the percentage of nevocytes with cytoplasmic immunoreactivity was also recorded in this study, with the same scale. Because of the obvious differences in immunoreactivity for the two spatially distinct cell populations within nevi, a separate epidermal and dermal score was recorded for all the tissue samples. These representation regions are illustrated in the haematoxylin and eosin sections shown in Fig. 1. Breast carcinoma tissue was included in each immunostaining batch to serve as an external positive control. Consistency in the degree of immunostaining was insured by using ER β -positive lymphocytes and sebocytes as internal positive control cell populations within each of the sections. Negative control slides (incubated without primary antisera) were also included in each immunohistochemical staining batch.

Statistical analysis

Epidermal and dermal scores were recorded for the nuclear and cytoplasmic staining for the six different nevus categories, and the mean and median staining averages were calculated (Table 3). As the data appeared to be non-normally distributed, statistical analysis was performed using the non-parametric Wilcoxin test to compare two paired groups and a P -value ≤ 0.05 was used to determine statistical significance.

Results

A total of 212 nevi were collected from 106 sex-matched patients in this study. A total of 17 normal nevi, 11 dys-plastic nevi and five congenital nevi were collected from pregnant patients and compared against 82 normal, 62 dysplastic and 35 congenital nevi from non-pregnant patients (Table 1). Our results confirmed that ER β is the predominant estrogen receptor in melanocytic cells from normal, dysplastic and congenital lesions in both pregnant and non-pregnant patients of child-bearing age.

Expression of ER α was not found in any of the melanocytic cells tested, although positive immunoreactivity was detected in the nuclei of sebocytes at the periphery of sebaceous glands, which served as an internal positive control within the adjacent dermis in majority of the samples (Fig. 1g).

Increased ER β immunoreactivity in pregnant normal nevi when compared with non-pregnant normal nevi

The relative numbers of immunopositive-stained nevocytes in 14 pregnant patients with 17 normal nevi were compared against 47 non-pregnant patients with 82 normal nevi (Table 1). Normal nevi in pregnant patients showed a strikingly significant greater nuclear immunoreactivity for ER β when compared with normal nevi from non-pregnant patients, when nevocytes were separately scored from both the epidermal ($P = 0.0054$) and the dermal ($P = 0.0013$) compartments (Figs 2 and 3b,d). An average nuclear staining intensity of epidermal nevocytes was 2.94 and dermal nevocytes was 3.35 for normal nevi in pregnant patients. This compares to the lower intensity of immunoreactivity in epidermal nevocytes (1.88) and dermal nevocytes (2.40) observed in normal nevi from non-pregnant patients (Table 3). These relative differences in staining intensities in the various regions are photographically depicted in normal nevi from non-pregnant patients in Figs 1b,d,e and 3a,c and in normal nevi removed from pregnant patients (Fig. 3b,d). In addition, increased cytoplasmic staining for ER β was also seen in both the epidermal ($P = 0.0036$) and the dermal ($P = 0.0073$) nevocytes from normal nevi in pregnant patients compared with non-pregnant patients (Table 3). Interestingly, while normal nevi from both pregnant and non-pregnant women were associated with increased nuclear staining in the dermally located cells, cytoplasmic staining was maximum in the epidermally located nevocytes regardless of pregnancy status.

Equivalent ER β immunoreactivity in dysplastic nevi from pregnant compared with non-pregnant patients

A total of seven pregnant women with 11 dysplastic nevi were compared with 36 non-pregnant women with 62 dysplastic nevi (Table 1). In all cases of dysplastic nevi, irrespective of their hormonal status, increasing histologic melanocytic atypia was associated with increased immuno-reactivity for ER β in nevocytes nested within the epidermis (Fig. 3i–l). No statistically significant increases in staining intensity were observed in dysplastic nevi from pregnant patients versus non-pregnant patients. Nuclear staining of nevocytes in the epidermal and dermal locations averaged 3.09 and 2.27 in dysplastic nevi from pregnant patients compared with 3.00 and 2.10 in atypical nevi from non-pregnant patients (Table 3). Cytoplasmic staining averaged 2.27 and 0.91 for epidermal and dermally located dysplastic nevi from pregnant patients compared with 1.76 and 0.79, respectively, in dysplastic nevi from non-pregnant patients. Dysplastic nevi from both pregnant and non-pregnant patients showed a distinctively higher level of ER β immunoreactivity in the nuclear and cytoplasmic staining of nevocytes that were positioned within the epidermal compartment (Fig. 3i–l).

Decreased ER β immunoreactivity in congenital nevi from pregnant versus non-pregnant patients

A total of two pregnant patients with five congenital nevi were compared against 17 non-pregnant patients with 35 congenital nevi (Table 1). Overall, congenital nevi tended to have decreased staining for ER β compared with normal and dysplastic nevi (Fig. 3e–h). Pregnancy was associated with a further diminution in ER β immunoreactivity as evidenced by lower nuclear staining (1.00 for epidermal and 2.00 for dermal) and cytoplasmic staining (0.20 for epidermal and dermal) when compared with the nuclear staining (1.66 for epidermal and 2.11 for dermal) and cytoplasmic staining (0.77 for epidermal and 0.66 for dermal) in congenital nevi from non-pregnant women (Fig. 3f,h; Table 3). However, this decrease in immunostaining observed in congenital nevi from pregnant women did not achieve statistical significance. Congenital nevi were associated with a considerable dermal-nevocyte population that tended to exhibit a decreasing gradient of ER β immunoreactivity with increasing dermal depth (Fig. 3e–h).

ER β immunoreactivity in congenital nevi compared with normal and dysplastic nevi from pregnant patients

Five congenital nevi from two pregnant patients were compared against 17 normal nevi from 14 pregnant patients (Table 1). These congenital nevi from pregnant patients showed significantly decreased staining compared with normal nevi removed from pregnant patients in both their nuclear staining ($P = 0.0211$, epidermal and $P = 0.0080$, dermal) as well as cytoplasmic staining ($P = 0.0407$, epidermal) with nearly significant staining within the dermis ($P = 0.0587$). Scores for congenital nevi in pregnant subjects averaged 1.00 and 2.00 for nuclear immunoreactivity in the epidermal and dermal compartments with 0.20 and 0.20 for cytoplasmic levels of staining in the epidermal and dermal locations, respectively. In addition, when the ER β immunohistochemical data observed in congenital nevi from the above pregnant women were compared with dysplastic nevi from pregnant women, the congenital nevi removed during the pregnancy also showed a decrease in nuclear and cytoplasmic immunoreactivity. However, this decrease in ER β staining was only significant for nevocytes confined to the epidermal layer ($P = 0.0163$ for both nuclear and cytoplasmic staining).

Discussion

We recently determined that ER β is the predominant estrogen receptor in melanocytes of melanocytic lesions from both male and female patients (25). The present study confirms and extends these findings by examining the potential responsiveness of nevi to the increased

estrogen levels that develop during pregnancy. Our cohort of patients was roughly age-matched based on our inclusion of women of child-bearing ages between 18 and 45 and was also sex-matched. Our results confirm that ER β , and not ER α , is the prevailing estrogen receptor in melanocytes. Nevus samples from pregnant women appeared to possess a variable capacity for estrogen responsiveness. Up-regulation of ER β was seen in normal nevi with pregnancy, no statistical change was observed in dysplastic nevi and surprisingly, down-regulation of ER β was observed in the five congenital nevi when compared with normal nevi in pregnant patients. The most profound increase in immunoreactivity for ER β was observed in normal nevi during pregnancy. Two notable patterns of immunoreactivity were evident. Firstly, the degree of immunostaining was highest in the nevi removed from the pregnant patients (in normal and dysplastic nevi). Secondly, the intensity of the staining was linked to the spatial location of the nevocytes.

Nevocytes exhibiting atypia displayed a positive correlation between the degree of atypia and the level of expression of ER β . This was observed in both pregnant and non-pregnant patients. We therefore found no relationship between pregnancy and the level of ER β -positive nevocytes when cellular atypia was present. Interestingly, dysplastic nevi in both pregnant and non-pregnant patients showed a higher level of ER β immunoreactivity, with presumed greater estrogen responsiveness, in nevocytes nested within the epidermis proper. This spatially distinctive pattern of staining observed only in dysplastic nevi was in concordance with our previous research (25). In our earlier study, we noted that ER β immunoreactivity of melanoma and dysplastic nevi was dependent upon the micro-environment surrounding the cells with the most intense staining for ER β associated with melanocytic cells closest to the epidermal surface (25). Our data suggest that cytologic atypia of nevocytes may impart such a profound effect on ER β expression that the changes induced by pregnancy are not detectable by the semi-quantitative methods used in our study.

As mentioned above, microscopic evaluation of the nevus samples revealed the presence of two distinct nevocyte cell populations that can be distinguished based on spatial considerations, the intensity of immunoreactivity for ER β , and cellular shape. Nevocytes located more superficially and along the dermal–epidermal junction appeared epithelioid in shape and regularly clustered into nests. These nevocytes tended to have the maximum increase in staining when associated with cellular dysplasia, regardless of pregnancy status. Nevocytes in the dermis are associated with a more spindle-shaped change in nevocyte morphology, which resemble neural or Schwann cells (29,30). Immunoreactivity for ER β was found to decrease with such deeply extending cells. In our study, this was most noticeable in large congenital nevi from either pregnant or non-pregnant patients that consisted of a predominant population of deep, dermally located cells. Our theory that melanocytic regulation and proliferation is influenced by the cellular micro-environment, particularly melanocytic–keratinocytic interactions, is supported by observations of similar distributions with other molecules. For example, cell adhesion molecules such as E-cadherin and β -catenin also show a loss of expression in nevocytes and melanoma as they extend deeply into the dermis (31–34). Whether nevocytes lose their cell–cell interaction and regulatory control mechanisms as they also lose the ability to respond to hormones such as estrogen should be the focus of future studies. Alternative regulatory explanations may also explain and contribute to the spatial differences in ER β receptor intensities observed in the present study. For example, certainly neural crest cell-derived melanocytes and the skin as a whole are all functional components of the systemic neuroendocrinology system (26). Thus, different elements in the skin, such as keratinocytes and deeper-placed adnexa, have shown the capability of steroidogenic production of cholesterol via precursors ranging from the transformation of 17 β -estradiol into estrone or through host manipulation of steroid influences mediated by the hypothalamo-pituitary-adrenal axis (35,36). Thus, the implications for our findings are not immediately evident.

Our analysis may be limited by certain features of the study design. This study contained both a retrospective and a prospective component. Hormonal status was inferred from the available history. Thus, we were forced to make a few assumptions. If a patient did not declare a pregnancy, we assumed that she was not pregnant. It is entirely possible that a few patients in our non-pregnant category were in the first trimester resulting in unknown errors of misclassification. Samples from pregnant patients were collected during the 2nd and 3rd trimester, presumably when estrogens are on the upswing or are peaking. We suspect that our pool of samples from non-pregnant patients may have been somewhat of a mixed population with regard to hormonal status. Because of incomplete histories on the retrospective biopsies, we were unable to control for exogenous hormone use, such as oral contraceptives or hormone replacement therapy or dietary supplements containing phytoestrogens. Future studies should include a study design where estrogen levels are measured at the time of biopsy of the nevus to adjust for these confounding factors and others such as drugs or botanicals that are known to be estrogenic. Ultraviolet light exposure (such as sun-exposure or tanning bed use) is another unknown factor because of lack of history. Melanocytic lesions have a known linkage between darkening following sun exposure and increasing numbers with increased sun exposure (29).

The implications of an altered ER β expression during pregnancy remain subject to much speculation. In other estrogen-responsive tissues such as uterus, mammary glands and prostate, ER β functions to inhibit cellular proliferation when stimulated by estrogen (34). The anti-proliferative effects of ER β appear to be counteracted by the opposite effects of estrogen on ER α . Therefore, cellular growth and proliferation versus inhibition occurs based upon the predominant estrogen receptor in the tissue (24,34). Whether ER β effects melanoma cells and melanocytes in similar ways to cell populations in the uterus, mammary glands and prostate in light of the lack of ER α is an ongoing investigation. Increasing evidence suggests the possibility that estrogen may play a protective role in women diagnosed with melanoma as women tend to have a better prognosis than men; particularly, in earlier stage melanomas (37-40). We note two reports where male gender was found to be an independent negative prognostic indicator for survival (37,40). Although the current concept most likely responsible for the sex difference in melanoma survival is attributed to less vigilance on the part of men, and more older men having nodular melanoma, estrogen may evoke an anti-invasive response in melanoma cells, a mechanism that could hinder dermal and metastatic potential (41). Thus, up-regulation of ER β may be the body's intrinsic control mechanism for preventing pro-oncogenic melanocytic progression in nevi when faced with increased cellular atypia or at times of elevated estrogen states such as pregnancy. Ultimately, loss of ER β expression and its presumed inhibitory effects may promote transformation into a melanoma, a key event in neoplastic progression. The skin has its own endogenous capacity to produce steroids such as estrogens; thus, this is another confounding variable that adds complexity to the interpretation of our data (35,36). Conversely, dysfunctional estrogen regulation may play an important role in the development of dysplastic nevi or increased tumor thickness in pregnant patients with melanoma. Further research into the downstream effects and cellular signalling within nevocytes in response to estrogen is needed.

In summary, our findings suggest that melanocytic cells within normal, dysplastic and congenital nevi possess variable capacities to respond to estrogen. Factors such as pregnancy and increasing cellular atypia appear to be associated with enhanced levels of ER β . The clinical implications of such altered ER β expression remains unknown. Our small prospective study points to the need for a larger, population-based study to examine the clinical significance of altered ER β expression in pregnant patients. Such a study will need to collect careful histories to capture details about cosmeceuticals or supplemental products that may contribute to the estrogen levels as well as the obvious hormonal drugs such as contraceptives or hormone replacement therapy. Such work will be essential for establishing a standard of care for pregnant women with varying melanocytic lesions.

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Abbreviations

ER β , estrogen receptor β ; ER α , estrogen receptor α ; PN, pregnant normal; PA, pregnant atypical; PC, pregnant congenital; NPN, non-pregnant normal; NPA, non-pregnant atypical; NPC, non-pregnant congenital; DNS, dysplastic nevus syndrome..

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Figure 1.

(a–c) Micrographs stained with H & E illustrate epidermal and dermal regions that were defined for scoring purposes. (a) Dysplastic nevus from the lower back of a 34-year-old non-pregnant woman. The upper bracket shows nests of nevocytes in close association with the epidermis (epidermal nevocytes). The lower bracket shows a few, scattered nevocytes in the dermis (dermal nevocytes). Scale bar = 75 μ m. (b) Normal nevus from the neck of a 35-year-old woman. Photo shows the rounded, nest-like morphology that was typical of nevocytes spatially positioned nearest the epidermis (epidermal nevocytes). Bracket indicates the epidermal region. Scale bar = 100 μ m. (c) Dysplastic nevus from a 25-year-old woman. Photo shows the scattering of the deep, dermally positioned nevocytes. Bracket indicates spatial location of the

dermal nevocytes. Scale bar = 125 μ . (d–f) Micrographs illustrate the spatial differences in morphology and ER β immunoreactivity of nevocytes from a normal nevus from a 39-year-old non-pregnant patient. (d, e) Show intensely positive cells for ER β in the upper dermis. Scale bars = 100, 75 μ . (f) In contrast, nevocytes deeply positioned in the dermis are more spindle-like and tend to lose their ER β expression. Scale bar = 50 μ (g). Photo illustrates negative ER α immunoreactivity in nevocytes (arrows) from a normal nevus from a 43-year-old non-pregnant patient yet ER α -positive nuclear immunostaining is observed in sebocytes adjacent to the lesion (arrowheads). Scale bar = 50 μ . Epi, epidermal layer; SG, sebaceous gland.

Figure 2.

Nuclear ER β nevocyte spatial staining pattern values. (a) Epidermal nevocytes: ER β staining levels in epidermal nevocytes in lesions in pregnant and non-pregnant patients. Nuclear ER β levels are significantly higher in normal nevi removed from pregnant patients (* $P = 0.0059$) when compared with normal nevi from non-pregnant women. Dysplastic nevi showed equally high ER β levels between the non-pregnant and pregnant patients. Congenital nevi showed a non-statistically significant trend towards a decrease in ER β in the pregnant patients when compared with non-pregnant women. Congenital nevi during pregnancy showed a decrease in nuclear staining for ER β when compared with pregnant patients with normal nevi (** $P = 0.023$). Congenital nevi during pregnancy had a statistically significant decrease in nuclear

staining for ER β when compared with dysplastic nevi from pregnant women ($^tP = 0.016$) Grey columns indicate non-pregnant women. Black columns indicate pregnant patients. Bars indicate the SEM. (b) Dermal nevocytes: ER β staining levels in dermal nevocytes in lesions in pregnant and non-pregnant patients. Higher staining for ER β is present in normal nevi from pregnant patients when compared with non-pregnant patients ($*P = 0.0014$). Congenital nevi during pregnancy showed a decrease in staining for ER β when compared with pregnant patients with normal nevi ($**P = 0.0085$).

Figure 3.

Immunostaining for ER β in nevocytic lesions. Normal nevi: (a, c) Photos show immunostaining from a normal nevus from a non-pregnant woman with cell populations showing both positive and negative ER β immunoreactivity. Scale bars = 100, 50 μ . (b, d) Photos show immunostaining from a normal nevus from a pregnant patient. Greater numbers of immunopositive nevocytes are observed in both the epidermal and dermally positioned nevocytes compared with 3a,c. Scale bars = 100, 50 μ . Congenital nevi: (e, g) Photos show immunostaining in a congenital nevus from the thigh of a 22-year-old non-pregnant woman. Large numbers of intensely positive ER β nevocytes are prominent (arrows). Scale bars = 200, 50 μ . (f,h) Show immunostaining in a congenital nevus from the breast of a 22-year-old

pregnant patient. The numbers of ER β expressing nevocytes diminish in comparison to non-pregnant patients. Scale bars = 200, 50 μ . Dysplastic nevi: (i, j) show immunostaining in a dysplastic nevus removed from the breast of a 23-year-old non-pregnant patient. Note the high degree of immunoreactivity for ER β in both the nuclear and cytoplasmic locations. Scale bars = 50, 75 μ . (k, l) Show immunostaining in a dysplastic nevus removed from the breast of a 42-year-old pregnant patient. Note the high degree of immunoreactivity for ER β in both the nuclear and cytoplasmic locations. Epi, epidermal layer. Scale bars = 50, 75 μ .

Table 1

Comprehensive listing of patients and nevi within this report

Nevus category	Patients (n = 106)	No of nevi (n = 212)
Pregnant normal	14	17
Pregnant dysplastic	7	11
Pregnant congenital	2	5
Non-pregnant normal	47	82
Non-pregnant dysplastic	36	62
Non-pregnant congenital	17	35

Table 2

Semi-quantitative scoring scale

Grade	Immunopositive cells (%)
0	<5
1	5–25
2	26–50
3	51–75
4	>76

Each lesion was independently scored in a blinded fashion by three independent observers. The percentage of estrogen receptor β was estimated after each observer viewed the entirety of each lesion.

Table 3

ER β staining averages

		Nuclear epidermal	Nuclear dermal	Cytoplasmic epidermal	Cytoplasmic dermal
Staining averages Pregnant normal	Mean	2.94	3.35	1.65	1.18
	SD	1.71	0.79	1.5	1.01
	Median	4	4	1	1
	75th Percentile	3	3	0	0
Pregnant dysplastic	Mean	3.09 (4)	2.27 (3)	2.27 (2)	0.91 (1)
	SD	1.22	1.49	1.49	1.04
	Median	4	3	2	1
	75th Percentile	2.50	1	1.50	0
Pregnant congenital	Mean	1.00	2.00	0.20	1.50
	SD	1.41	0.71	0.45	0.20
	Median	0	2	0	0.45
	75th Percentile	0	2	0	0
Non-pregnant normal	Mean	1.88	2.40	0.66	0
	SD	1.62	1.15	1.02	0.54
	Median	2	3	0	0.89
	75th Percentile	3	2	0	0
Non-pregnant dysplastic	Mean	3.00	2.10	1.76	1
	SD	1.12	1.50	1.48	0.79
	Median	3	2	2	1.07
	75th Percentile	2	0.25	0	0
Non-pregnant congenital	Mean	1.66	2.11	0.77	1
	SD	1.66	1.08	1.31	0.66
	Median	2	2	0	1.24
	75th Percentile	0	1	0	0
		3	3	1.5	1