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Premalignant and malignant skin diseases
Assessment of proliferation indexes

Malignant diseases, such as proliferative skin lesions are characterized by inappropriate cell increase. Activation of proto-oncogenes and inactivation of tumour suppressor genes are the harmful genetic events that are responsible for malignant transformation (1). Carcinogenesis in the skin is a complicated process characterized by the appearance of cells which have escaped from the normal growth control mechanisms, following in an increase of proliferative activity in the cells (2). The prevalent risk factors for skin cancer are exposure to ultraviolet (UV) radiation and fair skin that is receptive to sunburn. Increasing frequency of exposure, immune status, age, male gender and DNA repair disorders such as xeroderma pigmentosum also assist to increased risk (3). The association between UV exposure and cancer is powerful for SCC but less well-defined for BCC, as nearly one third of all BCC originates in anatomical sites receiving minimal UV exposure. Skin temperature may also be a risk factor. Cultured immortalized human keratinocytes have been shown to spontaneously transform to a tumorigenic phenotype when incubated at elevated temperature (4). Some investigations indicate that human papilloma virus infection may be a risk factor for skin cancer, though the relationship is not clear (5). Solid-organ transplant recipients are at extremely high risk for SCC (6). The long-term immunosuppressive therapy is probably the cause of the increased frequency of SCC in these patients, particularly in persons with chronic actinic damage (7).

The mechanism of UV-induced photo carcinogenesis appears to implicate the inactivation of the p53 tumour suppressor gene (8). Several studies have reported that p53 distortions play a critical role in the development and progression of various human malignancies. The p53 gene is involved in the regulation of cell cycle control and apoptosis. Mutations in this gene could presumably cancel p53 function, allowing accumulation of mutant cells due to loss of the UV-induced apoptosis of DNA-damaged cells (9). This leads to clonal expansion of p53-mutated keratinocytes, which could eventually acquire the second mutation that leads to the progression to multiple mutations. They can be accumulated. Therefore, this clonal expansion is regarded as a key initiating step in epidermal carcinogenesis (10). Precancerous dermatoses occur prior to development of epidermal malignancies (6). Because skin lesions are visible and easily accessible, skin cancer provides us with an excellent model for studying the development and growth of cancer in humans. Furthermore, the carcinogenic agents to the skin are well known. This is of special interest so far as UV radiation is the most important carcinogen and puts a specific signature on the DNA (10).

Actinic keratosis (AK), also known as senile or solar keratosis is the most common precancerous dermatosis and is attributable to UV radiation (11). This UV-induced lesion is usually seen as multiple lesions in sun-exposed skin in elderly persons. This sunrelated disease may develop into carcinoma *in situ* or invasive SCC. However, it is a controversial subject, based on the absence of a clear-cut distinction between AK and SCC (12). AK occur from proliferation of transformed

neoplastic keratinocytes limited to the epidermis and is characterized by thickened, scaly lesions that develop on the surface of the skin (13).

Histologically, there is cytological atypia in the basal/suprabasal layers. Occasionally the lesions show marked hyperkeratosis. Parakeratosis and dyskeratosis are also frequent features for this premalignant disease. Usually the elastosis is in the dermis too (14). AK can be destroyed and cured with cautery or cryotherapy.

Basal cell carcinoma (BCC) is approximately 80% of all non-melanoma skin cancers (3). These neoplasms, originally described by Jacob in 1827 (15), appear to originate from basal cells of the epidermis and occasionally those of the infundibular and outer root sheath of the hair follicles. These slow-growing tumours are locally invasive, rarely metastasize and can be cured at any stage if excised completely. However, morbidity can be high because these tumours are often disfiguring and located in facial areas.

Squamous cell carcinoma (SCC) is relatively common skin tumour which, if untreated, generally has a progressive clinical course with resultant extensive local destruction, metastasis, and even death (16). SCC arises from the epidermis and has an initial progression stage in which proliferation of the neoplastic cells is confined to the epidermis. With invasive SCC, masses of malignant keratinocytes extend through the basement membrane showing different levels of atypia. Having crossed over into the dermis they gain the ability to metastasize. Diagnosing invasive SCC with complete conviction is not always possible given that morphologically other lesions can mimic this cancer.

MATERIAL AND METHODS

Formalin fixed, paraffin wax embedded tissue (40 cases for each skin lesions) was retrieved from the archives at the Pathology Department of the Medical University of Lublin, Poland. Sections, 4-micrometer thick were cut coated slides and dried overnight at 55°C, dewaxed in xylene and rehydrated through industrial methylated spirits. Endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol. Sections, after deparaffinization, were routinely processed, inclusive of antigen retrieval according to manufacturer's prescription for immunocytochemical demonstration of expression of cell proliferation by staining for the KI67 antigen using the Monoclonal Mouse Anti-Human KI67 Antigen antibody for the MIB1 (dilution 1:100, DakoCytomation, Denmark). The MIB1 monoclonal antibody reacts with the KI67 nuclear antigen associated with cell proliferation and is expressed throughout the cell cycle (G1, S, G2, M phases) and is absent in resting (G0) cells (17). Bound antibody was visualised using the Dakocytomation Envision+Peroxidase Kit, diaminobenzidine was used as chromogen. Sections were lightly counterstained with Mayer's haematoxylin. KI67 (MIB1) positive cells were counted per 1,000 cells. The sections were examined at high power (X40) and 10 fields were chosen in the area showing most proliferation.

RESULTS

The results showed the range of KI67 expression of total area to be 4.00%–20.00% (mean 10.13%) in AK; 7.00%–50.00% (mean19.73%) in BCC and 9.00%–61.00% (mean 27.15%) in SCC. The mean, median and range of values for IP are shown in Table 1. The IP of keratinocytes as defined by KI67 expression was significantly higher in SCCs and BCCs then in AKs (p<0.0001). Significant differences were found in KI67(MIB1) staining between the IPs in SCCs and BCCs with greater value in the SCCs (p<0.0011). Test ANOVA and post-hoc Newman-Keuls test was using in our study. Figures A, B, C show specimens of KI67 immunostaining in AKs, BCCs and SCCs.

Table 1. AK, BCC and SCC proliferation indexes, scored per 1,000 cells, presented as mean (M), standard deviation (SD), range (min, max) and as median (Me)

	n	min	max	M	SD	Me	p
SCC	40	9.00	61.00	27.15	11.81	24.50	
BCC	40	7.00	50.00	19.73	11.74	14.50	< 0.0001
AK	40	4.00	20.00	10.13	3.62	10.00	

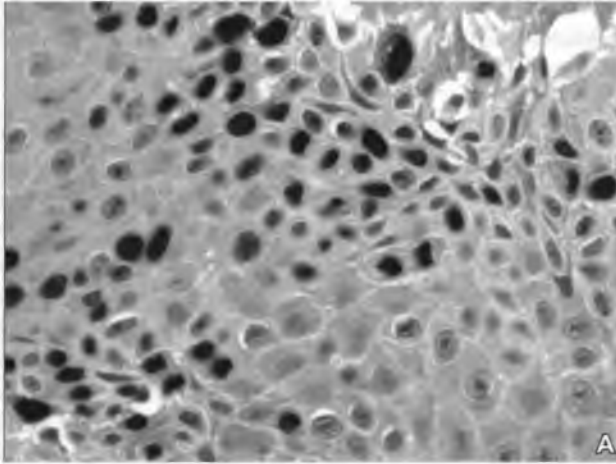Test ANOVA and post-hoc Newman-Keuls test was using in our study

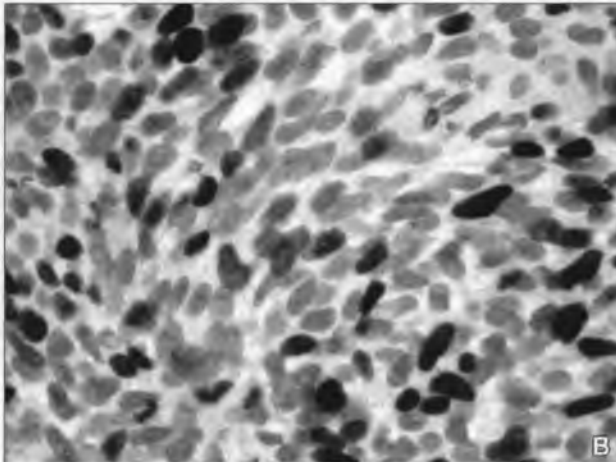Figure A. Specimen of KI67 (MIB1) staining in a AK (original magnification, X400)

Figure B. Specimen of KI67 (MIB1) staining in a BCC (original magnification, X400)
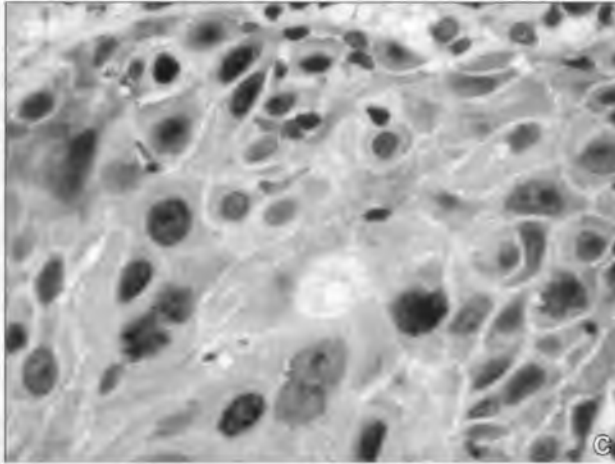
Figure C. Specimen of KI67 (MIB1) staining in a SCC (original magnification, X400)

DISCUSSION

In recent years several studies have examined proliferation in various human tumours for diagnostic and prognostic aims, demonstrating that cell proliferation markers can be potentially useful for the evaluation of tumours. In different malignant diseases MIB1 expression correlates with morphologic features of proliferation (18). Immunohistochemistry studies evaluating MIB1 expression in skin samples would also be useful. MIB1 expression varies through the cell cycle with increasing detectable antigen as the cell passes from G1 through S, G2 and M phases of the cell cycle respectively (19, 20). KI67 is an antigen associated with nuclear proliferation and is regarded as a useful marker for estimation of proliferation in malignant diseases.

We assessed KI67 expression using the MIB1 clone, a well-known marker of cell proliferation. In AK expression was seen in the basal/suprabasal layer. Some MIB1 positive nuclei were found in the lining epithelium of the hair follicles. AK showing an interrupted MIB1 presentation. In the BCC variable intensity levels of KI67 have been expressed more diffusely within the basal layers of the epidermis. In the SCC the expression was different. The expression limited to peripheral zones of the tumour fields was alternating with diffuse expression throughout the full lesion thickness. The common feature of all the slides with SCC was the total loss of polarity in the MIB1 staining pattern, following in a chaotic appearance.

We observed that the MIB1 expression seen in the SCC slides with the chaotic and diffuse nature of the pattern. The case in which morphologically AK can only be separated from SCC with difficulty can be beneficial additional method to differentiate these diseases.

Cutaneous BCCs and SCCs differ relatively to behaviour and metastatic potential. Why BCCs have a more advantageous course remains an enigma. Researchers have attempted to find an explanation for this. None of investigations of apoptotic indexes, cell proliferation or oncoprotein expression have given a satisfactory explanation for the difference between BCCs and SCCs (21). We observed a significantly higher IP in SCCs compared with BCCs. May be it is one of the factors underlying the more aggressive behaviour of SCCs. Of course, this difference with proliferation index in SCCs and BCCs may not be the only factor responsible for the different biological behaviour of this tumours. The correlation between increased cell proliferation and aggressive behaviour is present in a variety of cancers. We make no doubt that SCCs and BCCs are tumours with different phenotypes and this may also influence their behaviour (21).

Our observation showed that IP of keratinocytes as measured by KI67 expression was significantly higher in malignant skin diseases (SCCs and BCCs) than in premalignant skin lesion (AK). The importance of giving the correct diagnosis (especially in cases where the differential diagnosis includes premalignant and malignant abnormalities) is such that all available means should be studied and used for maximum certainty.

Our results may prove that MIB1 staining patterns should be studied prospectively for a potential role in differentiation between AK, BCC and SCC in more complex cases.

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SUMMARY

The aim was to compare differences in cell proliferation index (IP) between actinic keratosis, cutaneous basal and squamous cell carcinomas. Forty cases of actinic keratosis (AK), cutaneous basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) were retrieved from the archives. Sections, 4-micrometer thick, were immunostained with the mouse monoclonal antibody Ki67 (MIB1), positive nuclear staining was counted per 1,000 cells. The IP of keratinocytes was significantly higher in SCCs and BCCs then in AK. These results may prove helpful in histologic differentiation of these disorders. Difference in IP between these two tumour types may be one of the factors underlying the more aggressive behaviour of SCC.

Raki skóry i stany przedrukowe. Ocena indeksu proliferacji

Celem pracy było porównanie IP komórek w rogowaceniu słonecznym, podstawnokomórkowym i płaskonabłonkowym raku skóry. Bloczki pacjentów z rozpoznanym rogowaceniem słonecznym oraz rakami podstawnokomórkowym i płaskonabłonkowym (40 przypadków każdej jednostki nozologicznej) odnaleziono w archiwum. Pokrojone z nich skrawki o grubości 4 mikrometrów poddano barwieniu przy pomocy metod immunohistochemicznych z udziałem monoklonalnego przeciwciała MIB1. IP K167 wyliczano z liczby około 1000 komórek. IP keratynocytów był wyższy w rakach płaskonabłonkowych i podstawnokomórkowych w porównaniu z rogowaceniem słonecznym. Istotne różnice IP mogą być wykorzystane w rozpoznawaniu różnicowym tych chorób.