

The Influence of Pigment Transfer on the Risk of Developing Melanoma: The Significance of the Melanocyte 'Amputation Cycle'

Patrick A. Riley*

Totteridge Institute for Advanced Studies, The Grange, Grange Avenue, London N20 8AB UK

Abstract: It has been shown that cancer incidence is not only a function of the size of the population at risk but is strongly associated with the turnover rate of the tissue concerned. There is a strong negative correlation between melanoma incidence and the degree of skin pigmentation, and yet the melanocyte density is the same for all races. The proposal advanced in this communication is that the probability of undergoing malignant change is critically dependent on the melanocyte turnover and that this is regulated by the pigmentation process.

In melanocytes, the division rate is influenced by the process of pigment donation, probably by a mechanism whereby the continual cytoplasmic loss due to cytotrine transfer of melanosomes (termed the 'Amputation Cycle') inhibits replication. Consequently the turnover of melanocyte stem cells in heavily pigmented epidermis will be diminished, and this is held to account for the strong negative correlation between the degree of skin pigmentation and melanoma incidence.

Keywords: Epigenetic, progression, melanoma, cytotrine transfer, stem cell proliferation.

INTRODUCTION

The well-established age dependence of the majority of human cancers has broadly been interpreted as a stochastic phenomenon in which the emergence of a malignant variant of a population of cells at risk is the result of a series of random and independent events. In adults the age-specific incidence of various cancers, and therefore the presumed initiation rates of these malignancies, is proportional to about a sixth power of age. The general opinion regarding the nature of the stochastic events has favoured the accumulation by a single cell of a number of somatic mutations [1,2]. However, the mutation rates necessary to generate the observed age-specific incidence of cancer in man are in the range 10^{-2} to 10^{-4} per gene per cell per year [3] and are thus several orders of magnitude greater than the presumed mutation rates estimated from the observed frequency of germ-cell mutations which lie in the region of 5×10^{-8} to 4×10^{-6} [4,5].

The apparent incompatibility of the enhanced rate of genetic variation exhibited by pre-malignant and malignant cell populations with the estimates of the somatic mutation rate has been noted [6-9], and a number of ways in which the somatic mutation rate might be accelerated have been suggested. Proposed mechanisms include the acquisition of DNA repair deficiency, increased sensitivity to potential mutagens through diminished detoxification ability, and the

intrinsic generation of mutagenic species through deranged metabolism [10]. It has been proposed that reactive oxygen species have mutagenic properties and metabolic derangements leading to chronic oxidative stress increase the mutation rate in pre-malignant cells. Another view is that, as S-phase cells are more susceptible to DNA damage, increased proliferation might account for the raised mutation rate, although the enhancement is relatively small [11]. A proposal by Holliday [12] invoked raised susceptibility of methylated segments of the genome to DNA damage and error-prone repair, thus implicating epigenetic mechanisms in carcinogenesis. However, none of these explanations have seemed adequate to account for the high genetic variability exhibited by pre-malignant and malignant cell populations [13,14].

A second difficulty inherent in the multistage carcinogenesis concept is that the somatic mutation model does not take into account evidence which suggests that the process can be divided into an initial carcinogen-requiring stage with a subsequent phase of development (progression) that does not require the presence of an initiating carcinogen [15] and it has been suggested that some of the stages of carcinogenesis are not due to mutations [16,17] although the nature of these non-mutational events was not identified.

However, it has recently been suggested that the progression phase of carcinogenesis is due to faulty copying of the epigenetic pattern in the initiated cell and its progeny [18,19], a proposal that also solves the problem of the apparent high somatic mutation rate. In essence the theory proposes a two-step

*Address correspondence to this author at the Totteridge Institute for Advanced Studies, The Grange, Grange Avenue, London N20 8AB UK; Tel: (+44) 20 8445 5687; E-mail: rebc900@ucl.ac.uk

carcinogenesis in which the initiating lesion consists of one or more mutation(s) that cause faulty copying of the epigenetic pattern which is responsible for the reproduction of the differentiated cellular phenotype. Such a lesion would result in high variability in the gene expression in the division products of the affected clone which would give the outward appearance of a raised mutation rate. Since an inherited defect in the vertical transmission of the differentiated cellular genome due to failure of fidelity of epigenetic copying will be manifested only when the cells divide, the extent of the variability in the affected population will reflect the proliferation rate. Thus, the probability of acquisition of the malignant phenotype will be a function of (a) the total size of the population at risk of the initiating mutation(s) and (b) the proliferation rate of the stem cells that sustain the differentiated lineage. This relationship has been elegantly demonstrated for a wide range of tissues by Tomasetti and Vogelstein [20]. Applying this principle to the biology of melanoma permits a possible explanation of the racial difference in melanoma incidence in populations occupying the same environment.

SIZE OF POPULATION OF CELLS AT RISK OF INITIATING MUTATION

With regard to the size of the total population at risk of the initiating mutation(s) it has been established that the normal melanocyte density in the skin is the same for all races [21]. It would be anticipated that the total melanocyte population would be increased by the presence of moles and there is evidence that melanoma incidence is increased in individuals with many melanocytic naevi [22-24]. However, this relationship does not explain the racial differences in

melanoma incidence and thus any difference must relate to the proliferation rate of the melanocyte stem cells. The argument advanced here is that there is a distinction in the turnover rate of epidermal melanocytes that is based on their physiological function.

CYTOCRINE PIGMENT TRANSFER AND LOSS OF MELANOCYTE VOLUME

The major mechanism of pigment transfer involves the phagocytosis by epithelial cells of melanosome-containing melanocyte dendrites (see review by Van Gele & Lambert, [25]). The transfer process involves protease activated receptor-2 (PAR-2) which is expressed on the keratocyte surface and enhances phagocytosis. PAR-2 expression is higher in dark-skinned individuals and is stimulated by UV irradiation [26]. The process is termed cytocrine transfer and consists of the transfer of sections of the melanocyte cytoplasm containing melanised melanosomes (Figure 1) with consequent loss of cytoplasmic volume.

EFFECT OF CYTOPLASMIC LOSS ON PROLIFERATION RATE

It is known that in mammalian cells there exists a size checkpoint which regulates entry in to S-phase [28-30]. The removal of melanocyte cytoplasm may influence growth control indirectly through loss of cell surface receptors or by reduction in calcium sequestering organelles, in particular melanosomes. Other relevant factors may include Mitf [31] or eIF3 [32], although the detailed molecular mechanisms that delay proliferation of undersized cells remain obscure [33]. However, the existence of a cellular regulatory mechanism which delays mitosis of small cells will

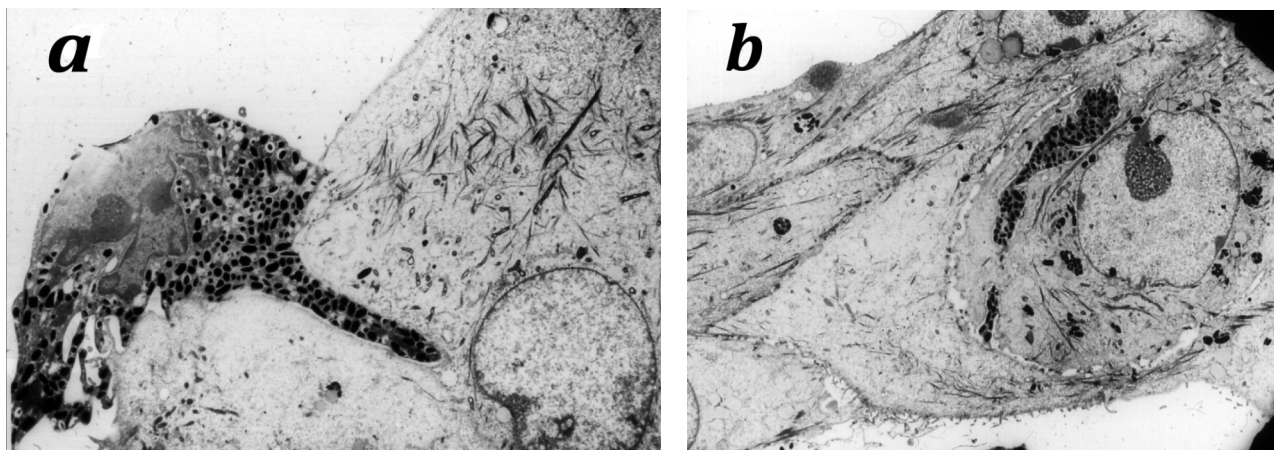


Figure 1: Electron micrographs of a co-culture of guinea pig melanocytes and keratocytes showing: (a) the phagocytosis of a pigment-laden melanocyte dendrite; and (b) engulfed portions of melanocyte within the keratocyte cytoplasm (from [27]).

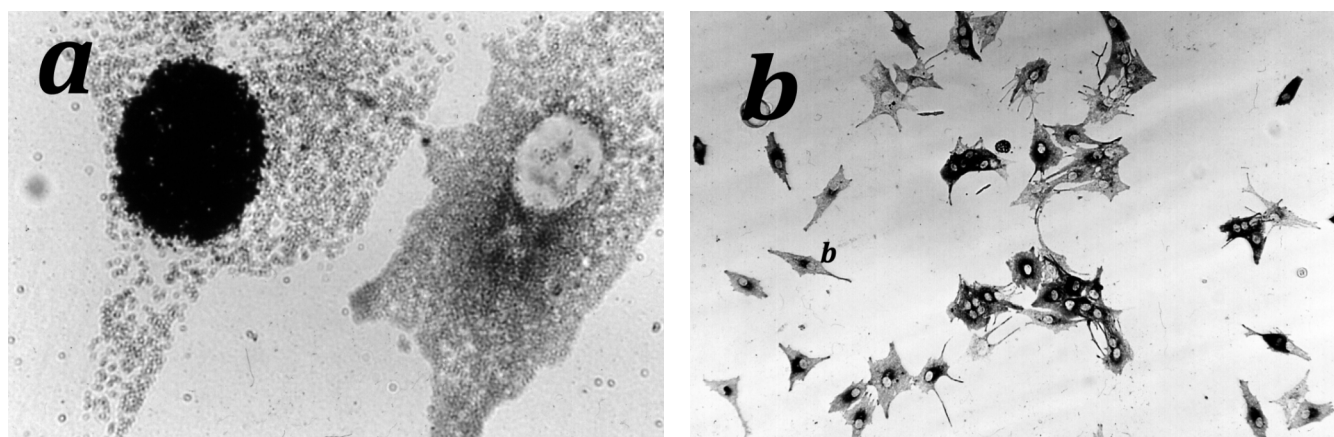


Figure 2: Proliferation of pigmented melanocytes under invitro conditions in the absence of recipient cells. The image (a) shows an autoradiograph of two adjacent melanocytes laden with pigment granules with one of the nuclei enlarged and overlain with silver grains marking the uptake of H^3 -thymidine showing DNA synthesis. The transmitted light micrograph (b) shows a proliferating culture of melanocytes.

therefore tend to restrict the proliferation of melanocytes that are active in pigment transfer. Thus, it is proposed that melanocyte proliferation is inhibited by the loss of cytoplasmic volume inherent in the cytokine transfer of pigment to the adjacent cells and that the turnover of melanocytes will be inversely dependent on the degree of pigmentation.

If pigment transfer does not take place, melanocyte proliferation can occur, as shown by isolated cultures where there are no recipient cells (Figure 2), or under conditions in which cytokine transfer is inactive or prevented.

It has been suggested that this regulatory phenomenon provides an explanation for the formation of pigmented naevi [34] since there are no suitable acceptor cells in the dermis and melanocyte proliferation is not inhibited by loss of cytoplasm due to pigment transfer.

The proposed regulatory effect of pigment donation on melanocyte proliferation can be summarised in terms of an amputation cycle (Figure 3). The operation of this regulatory process explains why melanocyte proliferation is infrequent in sites where melanogenesis and pigment distribution is high, and predicts an inverse correlation between the extent of melanin synthesis and epithelial pigmentation and the rate of melanocyte proliferation.

MELANOCYTE TURNOVER AND MELANOMA INCIDENCE

The explanation of the differences in melanoma incidence turns on the significance of the turnover rate in the carcinogenic process. The epigenetic model of

carcinogenesis [18,19] proposes that the progression phase is brought about by the failure of the epigenetic mechanism to accurately copy the restricted gene expression pattern of differentiated cells. Consequently, since the defective copying of the epigenetic pattern of gene expression generates cells possessing a divergent range of properties, the probability of the emergence from a stem cell bearing the initial carcinogenic mutation of a sub-clone possessing malignant properties will be dependent the rate of turnover of the cells. Hence, cells in which the turnover rate is accelerated will be more likely to result in malignancy whereas cells with low turnover rate are less likely to progress to frank malignancy. In general, it

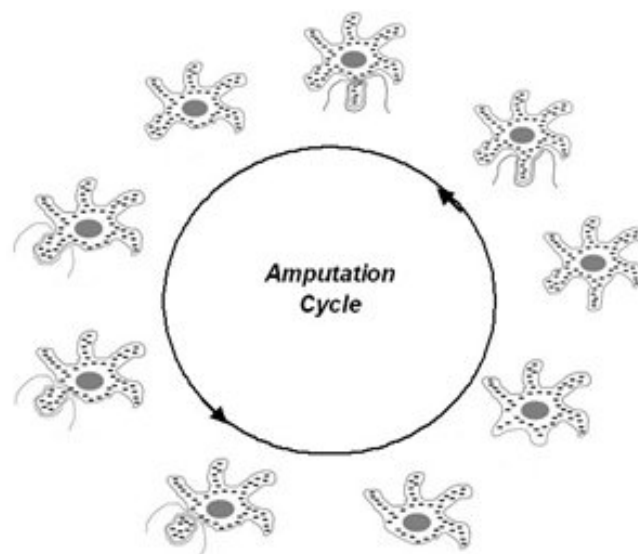


Figure 3: Schematic outline of the 'Amputation Cycle' showing pigment donation by cytokine transfer resulting in loss of cytoplasmic volume, thus preventing melanocyte proliferation.

is known that cancers arise in so-called 'labile' cell populations such as epithelia in which proliferation is rapid, less frequently in 'stable' populations that rarely undergo mitosis, and not at all in non-proliferative cell populations. Therefore, on the basis of the argument that a high rate of pigment production inhibits melanocyte proliferation, melanoma would be expected to be rare in heavily pigmented individuals, as observed for the racial difference in melanoma incidence. The SEER data [35] show that there is a marked difference in cutaneous melanoma incidence between whites and blacks resident in the same environment (Table 1). Moreover, the functional hypothesis advanced here also explains the significantly reduced melanoma risk in whites with heavy occupational sun exposure associated with increased pigmentation, with an odds ratio of 0.86 [36].

Table 1: Racial Difference in Cutaneous Melanoma Incidence from the US Cancer Registry. The Data Show the Mean Incidence Per 100,000 Standardised Population over the Period 1975-2012

	Male	Female
Whites	24.48	16.88
Blacks	1.19	0.92

MELANOCYTE PROLIFERATION IN UNPIGMENTED SITES

This argument would not apply to regions such as the palms, soles and mucous membranes where there is little epithelial pigmentation. Indeed, epidemiological data shows that the melanoma incidence in these unpigmented sites is racially equivalent [37-39].

The control of melanocyte proliferation in unpigmented sites is not clearly understood. It is possible that epidermal trauma could initiate melanocyte proliferation through the action of local hormonal signals. Melanocyte growth is locally controlled by a group of paracrine factors produced in the skin, including bFGF/FGF2, HGF/SF, M/SCF, endothelins and MSH [40]. Hence, trauma to epidermal cells might be sufficient to engender a local stimulatory response and could plausibly be viewed as indirectly increasing melanocyte turnover and hence the probability of malignant transformation in regional melanocytes. Such a proposal, that melanoma incidence in unpigmented sites is indirectly related to epidermal damage, would go some way towards explaining the association of plantar melanoma with

barefootedness [41], and is consistent with the association of melanoma incidence with epidermal trauma associated with sunburn [36].

COUNTERARGUMENTS

A confounding factor with regard to the argument outlined above is the possibility that ultraviolet radiation plays a causal role in melanoma and that pigmentation has a photoprotective action. Although strictly comparable incidence data by ethnicity for non-melanoma skin cancer is scarce, it is recognised that non-melanoma skin cancer is less common in pigmented races and it might be argued that the low incidence of melanoma in blacks is simply a consequence of the UV protection afforded by melanin. This is difficult to refute although ethnic comparison of the incidence of melanoma and basal cell carcinoma, which is considered to be UV-induced, show that there is an order of magnitude difference in susceptibility in environmentally equivalent populations. Data from the Kenya Cancer Registry [42] show the race-specific mean annual incidence rates of BCC (per 10⁵ population) were 5.85 for Caucasians and 0.0065 for Africans, i.e. a 900-fold difference. This contrasts with the data for melanoma incidence in the USA [35] which show an approximately 20-fold difference between whites and blacks (see Table 1).

Another interesting observation that favours the melanocyte turnover argument is that melanoma is rare in patients with albinism [43], whereas basal-cell and squamous cell carcinomas are common [38]. This cannot be explained on the basis of susceptibility to UV mutagenesis, but follows from the inhibition of melanocyte proliferation by the amputation cycle, since melanosomal transfer is not affected by lack of tyrosinase activity [44].

CONCLUSION

In general, the overall probability of the emergence in a population of cells of a clone possessing malignant properties is influenced by a combination of (a) the extent of exposure to a mutagen or equivalent initiating stimulus; (b) the size of the population at risk; and (c) the rate of turnover of the cells.

Malignancy does not arise in non-proliferative tissues, and in proliferative cell populations the cancer risk reflects factors influencing the turnover rate. It is argued here that the physiological activity of melanocytes modifies their turnover rate and that this

accounts for the relatively low incidence of melanoma in highly pigmented individuals.

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