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Melanoma and the Problem of Malignancy

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RILEY, P.A. *Melanoma and the Problem of Malignancy*. Tohoku J. Exp. Med., 2004, **204** (1), 1-9 —— Of the overt biological properties exhibited by malignant cells two appear to command particular attention; these are (1) the transmigratory ability which empowers these cells to invade surrounding tissues and results in their metastatic and destructive potential, and (2) their ability to evade detection by the immune system of the host. Both of these characteristics may well involve several disparate mechanisms. However, it may be that there are some metabolic features that are common to malignant neoplasms which could go some way to explaining one of these behavioural anomalies. It is proposed that abnormalities of oxidative metabolism of cancer cells, resulting in the generation of reactive oxygen species, are responsible for the inhibition of the functions of vicinal antigen-presenting cells and, thus, the failure of the immune system to recognize tumour-specific antigens likely to be expressed by malignant cells as part of their transmigratory capability. ——— malignancy; carcinogenesis; immune surveillance; Langerhans cells; reactive oxygen species; minipig melanoma

Despite over a century of investigation and many sophisticated advances in the understanding of living systems the problem of cancer remains a serious source of therapeutic difficulty as well as an intellectual enigma. As Mitchell (1971) was to remark some time ago: "Cancer, if curable, why not cured?." In this paper I attempt an exploration of the general properties of malignant cells in the light of the characteristics exhibited by melanoma. Principally, I shall try to relate to human malignancy but I include in my ambit some examples taken from melanoma in animals. To what extent this approach is justified may be debatable but I hope that the knowledgeable and critical reader will permit the development of the general argument.

Definition of malignancy

Let us begin by reaffirming the essential pathological description of a tumour (due to Willis 1967) as composed of a viable population of cells capable of proliferation. In addition to this, a malignant tumour (as opposed to a benign neoplasm) exhibits the ability to invade (i.e., to transgress the normal tissue boundaries of the adult organism) and, in principle, to metastasize

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(i.e., to form colonies in new locations in the host). These latter properties are of great interest since they are at the root of the destructive potential and the clinical threat posed by cancer cells.

Etiology and pathogenesis of cancer

The etiological basis of cancer embraces a wide field and is by no means uniform; different malignancies being associated with specific environmental hazards (as in the case of cigarette smoking and carcinoma of the bronchus), but the general pathogenesis can fairly accurately be described as somatic mutations resulting in a heritable anomaly of growth in the affected clonal progeny. Although the details differ to some extent in different cases, the process leading to malignancy may be divided into two phases: (a) initiation; and (b) promotion. This posits an initial somatic mutation that sets the process in train and which permits a further series of mutations to occur resulting finally in the acquisition of the cardinal properties characteristic of cancer cells.

Initiation as the induction of a hypermutable clone

It has been proposed that the initiating mutation is one that increases the subsequent mutation rate in the affected cells and their progeny (Loeb et al. 1974; Loeb 1991; Riley 1982, 1990) and it has been noted for a long time that cancer cells display great phenotypic variability which Nowell (1976) ascribed to an enhanced rate of intraclonal evolution compatible with raised mutability. Indeed, the phenotypic anomalies of malignant cells enable their identification by cytology.

If it is assumed that, in general, there is no selectivity in which parts of the genome are affected by the raised somatic mutation rate then there is a great range of abnormalities that may arise, most of which are unrelated to properties of the cell that render the behaviour malignant. Moreover, the vast majority of mutations are likely to be deleterious, resulting in metabolic derangements that make the mutant cells less viable and less proliferatively efficient; a scenario that is, in effect, accelerated senescence. Perhaps included in the senescent changes are such phenomena as the loss of telomerase activity. Obviously, part of the clonal selection process in the genesis and progression of malignant tumours involves the elimination of defective sub-clones. The in vitro mirror of this selection is known as "immortalization."

However, not all mutations will be damaging to the efficiency of cells, and certain genetic alterations may confer advantage. Perhaps the most obvious of these changes are those affecting signal transduction that result in diminished reliance on external growth factors and an element of proliferative autonomy which characterises many benign neoplasms.

At our present stage of knowledge it is clear that there are many genetic abnormalities in cancer cells and it is not certain which ones are crucial in the expression of malignancy but it can be assumed that several genes have to be affected otherwise the general cancer incidence would be higher with a less marked age-dependence. In addition, there seem to be at least some genes that are specifically involved in the genesis of certain malignancies but it is by no means clear whether there is a definitive set of genetic changes that comprises the necessary and sufficient condition for malignancy.

The question of whether specific genetic changes are correlated with specific types of malignancy is implied by familial susceptibility to develop certain cancers and the discovery of some genes that are regarded as susceptibility genes. In the case of melanoma there are examples such as the role of CDKN2 in the familial atypical mole syndrome (FAM syndrome) (Gruis et al. 1995). In most cases the role of the susceptibility genes and their mechanism of action is not known but in some instances there is a connection with mutagenesis. For many years it has been recognised that certain DNA repair deficiency syndromes are associated with increased cancer risk. These syndromes, of course, involve germ-line mutations and the abnormalities are expressed in all somatic

cells. However, the increased risk is confined to cells that are exposed to the damaging stimuli the effects of which are normally abrogated by the repair mechanism in question. An example is xeroderma pigmentosum, a genetically complex abnormality of repair of pyrimidine dimers, which is associated with increased risk of skin cancer because this type of DNA damage is characteristically produced by UV radiation (Kraemer et al. 1994).

An interesting proposal with regard to melanoma susceptibility has recently been made in connection with dysplastic naevus syndrome. It has been shown that the melanocytes present in these naevi differ from those in normal skin in that they synthesize a greater proportion of phaeomelanin. Compared to the usual black melanin (eumelanin) pheomelanin contains more sulphur which is incorporated in the form of cysteine at an early stage in pigment synthesis. Pavel and coworkers (2004) have suggested that this may result in a relative deficiency of intracellular thiols (such as glutathione) with the consequence that these cells are less able to deal with oxidative stress and thus more likely to suffer oxidative damage to sensitive structures such as the DNA. Hence, the dysplastic naevus melanocytes would be expected to manifest a raised somatic mutation rate. This is a clear example of a genetic lesion that has a cell-type specific effect since it is confined to melanogenic cells.

Two cardinal properties of malignant cells

Transmigration As mentioned above, one of the requirements *sine qua non* of malignant behaviour is the ability to transgress tissue barriers (Riley 1985). Although this behaviour is confined in the adult to specialised cells, such as leucocytes, it is a normal feature of embryonic cells many of which have complex migratory pathways during development. An interesting example of this migratory behaviour is manifested by neural crest cells which give rise, *inter alia*, to melanocytes. It is not impossible to entertain the notion that the inappropriate re-expression of this embryonic migratory potential could be a component of the malignant syndrome if activated in the adult organism. Were this to be the case it might account for the differences in the invasive and metastatic potential of cancers arising from different tissues. Certainly, melanomas possess impressive invasive capability and it is well recognised that different cancers exhibit different patterns of distant metastasis. To some extent, this latter property is reliant on the suitability of the microenvironmental niche presented by different tissues since the haematogenous distribution of malignant cells is thought to be fairly homogeneous. A similar conclusion can be drawn from the examples of dormancy, where metastatic cells are prevented from proliferating by non-optimal local conditions of nutrition.

Evasion of immune surveillance

With regard to the characteristics of malignancy, in addition to viability, proliferation and migratory ability it is necessary to consider another important aspect of cell behaviour. If, as we have argued, malignant (and pre-malignant) cells have a raised somatic mutation rate it is likely that many genes that are not directly implicated in the malignant transformation will be affected. Two consequences follow from this: (1) deleterious mutations affecting the house-keeping genes of the cell will render the cell less viable and less reproductively competitive (i.e., will be manifested as accelerated ageing); and (2) mutations affecting structural components of cells will result in the generation of neoantigens. Whilst some of these may be cryptic, it is likely that a proportion will be associated with a cell surface presentation and thus attract the attention of the immune system. Yet, one of the remarkable features of cancer cells is that they appear to a large extent to escape immune surveillance. Therefore, a further characteristic would appear to be evasion of immune detection.

In experimental models of melanoma there are some examples of the crucial importance of this latter characteristic. In a minipig melanoma

model (see below) it has been shown that devitalization by ligating the blood supply of a single lesion results in the regression of all tumours whereas excision of a single lesion fails to have a similar effect. This observation suggests that once the immune system has been primed the tumour antigens are recognized and the malignant cells destroyed but that fully perfused tumour tissue prevents the initial recognition. It is tempting to propose that there is a metabolic feature of malignant cells that interferes with the process of antigen presentation. The details of such a mechanism remain to be untangled but the fact that hypoxia appears to interrupt it suggests that it involves oxidative metabolism in some manner.

Oxidative stress and the failure of immune surveillance in cancer

The basis of the analysis attempted in this communication is Burnet's proposal that one of the conditions necessary for the emergence of malignancy arises from the failure of "immune surveillance" by the host (Burnet 1970). The reasons for adopting this hypothesis may be summarised as follows:

1. The cells comprising malignant tumours have a greater degree of phenotypic heterogeneity than those of normal tissue. This appears to reflect genetic instability and is associated with the possession of diverse antigenic profile including neoantigens (so-called "tumour-specific" antigens). Human tumour antigens recognised by T-cells have been recorded (Boon and Vanderbruggen 1996; Kawakami and Rosenberg 1997).

2. Despite this characteristic, malignant cells are able to survive and proliferate, not only in the primary site that marks the clonal origin of the tumour, but also in distant sites of metastasis. This argues strongly against any significant immune restriction or containment of antigenically anomalous cells.

3. In certain cases of "spontaneous" regression of malignant tumours, which has been well-documented for melanomas both in animal models and in human disease, there is evidence of an immunological response comprising notably T-cell attack on the malignant cells (McGovern et al. 1983; Mackensen et al. 1994). Thus, the deviant cells are killed by an immunological mechanism (Pandolfino et al. 1992; Darrow et al. 1996) if an immune response is mounted by the host.

4. In the light of this it has to be concluded that the tumour antigens are recognised by the killer lymphocytes if they are suitably primed, so the failure of an immune response would appear to be due to the failure of the priming step.

5. Current opinion in immunology posits that initiation of an immune response to an antigen involves the mediation of a specialized group of antigen-presenting cells comprising a set of dendritic monocytes, including Langerhans cells. Langerhans cells are the rapporteurs of new antigens in epithelia and are prominent in the epidermis. Their function appears to involve the uptake of antigenic material, which is transported to the regional lymph nodes where antigen-presentation to lymphocytes takes place.

6. Therefore, if conditions in the region of the tumour were to exist that inhibited Langerhans cell function, abnormal cells manifesting neoantigens would fail to be recognised, i.e., there would be a failure of immune surveillance. The possible derangements of Langerhans cell function include: (a) failure to enter the affected region; (b) failure of survival of Langerhans cell in the vicinity of tumour cells; (c) inhibition of some aspect of the uptake, transport, or presentation of antigens by Langerhans cells.

7. Consequently, an attractive hypothesis to account for the failure of immunological detection of deranged cells is that cancer cells emanate one or more inhibitory signal(s) that interfere with these processes and, thus, result in diminished or absent antigen presentation.

8. Dendritic cells expressing the cutaneous leukocyte antigen (CLA +) enter the skin as Langerhans cell precursors. Epidermal entry by these chemokine receptor-expressing (CCR6 +)

dendritic cells is stimulated by macrophage inflammatory protein (MIP-3a) generated by keratocytes (Charbonnier et al. 1999) and it is possible that this step is repressed by a product of malignant cell metabolism.

9. Little is known about the processes that control the uptake of antigens, but maturation of Langerhans cells involves the conversion of CCR6 to CCR7 expression which encourages the emigration of the dendritic cells to the regional lymph nodes under the stimulation of secondary lymphoid tissue cytokine (SLC) (Charbonnier et al. 1999). The stimuli for the conversion of CCR6 to CCR7-expression include tumour necrosis factor (TNF) and lipopolysaccharide (LPS) (Cumberbatch and Kimber 1992; Roake et al. 1995).

10. Factors regulating the survival of Langerhans cells are largely unknown. However, it is known that Langerhans cell exhibit marked radiosensitivity (Peckham and Riley 1966), and UVA-induced immunosuppression (Kripke 1984) has been shown to be associated with dendritic cell depletion of the irradiated tissue. Moreover, UV-induced depletion of Langerhans cells in the epidermis has been shown to result in suppression of the contact hypersensitivity response (Odling et al. 1987).

11. A plausible mechanism whereby malignant cells escape immune detection invokes oxidative stress. Specifically, this hypothesis is that malignant (and pre-malignant) cells possess deranged oxidative metabolism, which generates reactive oxygen species (ROS) that prevent the survival or function of antigen-presenting (dendritic) cells in their vicinity.

12. It would be predicted, therefore, that deprivation of the blood supply to a malignant tumour, which would result in the failure to generate ROS, would have the effect of enabling the proper function of vicinal dendritic cells (Langerhans cells) and thus the mounting of an immune response to the malignancy. Such a phenomenon has, indeed, been observed, and an animal model of the process has been carefully described.

The argument advanced here, which is summarized in Fig. 1, may be generally applicable to malignant tumours. It is recognized that the susceptibility of dendritic cells to tumour-induced apoptosis reduces their efficacy in the host defences against cancer (Kurabayashi et al. 2004). Perotta et al. (2004) have shown, in a murine model of melanoma, that prior ex-vivo treatment of dendritic cells with nitric oxide resulted in an enhanced response rate when these cells were injected into B-16-bearing mice. Nitric oxide preexposure prevented the tumour induced expression of Bcl-2, Bax, Bcl-xl, activation of caspase-9 and reduction of mitochondrial membrane potential. Interestingly, there is an inducible nitric oxide synthase present in dendritic cells (Cruz et al. 2004) which is activated by contact with the contact sensitizer, nickel sulphate, which may be important in protecting these cells from oxidative stress. However, this pathway may be inhibited by peroxynitrite, which was found in another system to prevent dendritic cell differentiation (Fernandez-Ruiz et al. 2004), and superoxide is the main agent for the conversion of nitric oxide to peroxynitrite. It would seem that reactive oxygen species have several actions that render them mediators of apoptosis and loss of dendritic cells, as indicated schematically in Fig. 1(B). Kuchel et al. (2003) concluded that ROS are involved in the UVR-induced loss of Langerhans cells from the epidermis. Since ROS are derived from oxygen by partial metabolic reduction it would be anticipated that deprivation of the vascular supply to a tumour will result in the cessation of ROS production and the survival of dendritic cells.

Minipig melanoma

Perhaps the most readily accessible experimental model of cancer in which the devitalization approach has been examined is that of melanoma regression in the Minipig, as mentioned above. Melanoma appears to be an example of a malignancy that occurs relatively early in life.

A

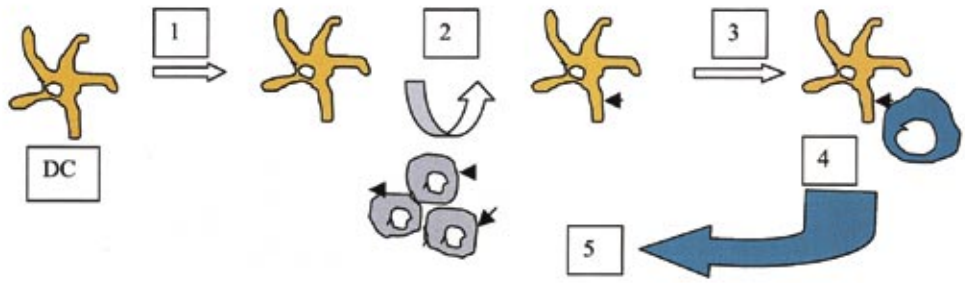
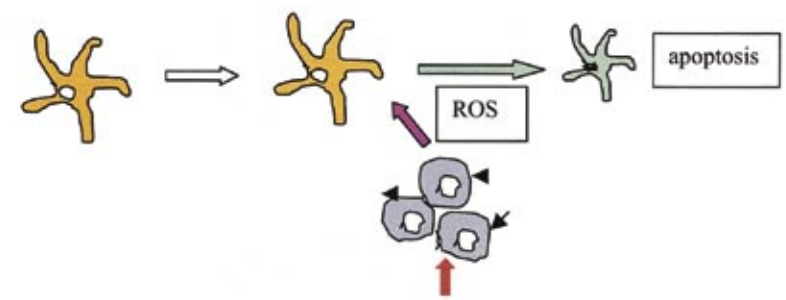


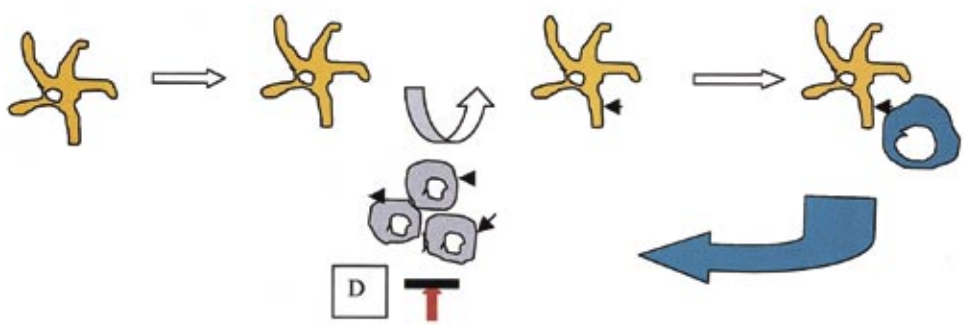
Fig. 1. A: Schematic outline of antigen uptake and presentation by dendritic cells. Dendritic cells (*DC*) enter the target tissue (*1*) and (*2*) take up neoantigens (▲) expressed by the invading cells. They leave the tissue (*3*) and proceed to the regional lymph nodes where antigen presentation occurs (*4*) resulting in an immunological response (*5*).

B



B: The oxygen supply to the tumour cells (red arrow) enables the production of reactive oxygen species (*ROS*, purple arrow) by malignant cells which initiate apoptosis in dendritic cells thus preventing antigen uptake and presentation.

C



C: The effect of devitalization (*D*), in depleting the oxygen supply to the tumour, restores the normal antigen detection and presentation mechanism resulting in an immune response to the tumour cells.

Unlike retinoblastoma it does not appear, in general, to arise during development but falls into the category of childhood (or young adult) malignancies. Among the animal models that have been examined perhaps the most relevant set of melanomas are those that occur in swine. These include the Sinclair swine of which a high proportion are either born with melanomas or develop them soon after birth. These melanomas metastasize and kill some of the animals. In others there seems to be the development of an immune response, initially signalled by depigmentation of certain tumours, with ultimately complete regression of the metastatic lesions together with loss of surface pigment due to the elimination of melanocytes from the skin. Where regression occurs there is evidence of a T-cell response to melanocyte-specific antigens. Thus the outcome is dependent on the recognition of melanocyte-specific antigens and hence an example of immune surveillance in relation to cancer. Recently a porcine model of melanoma with similar features has been described (Horak et al. 1999; Borovansky et al. 2003) in which the pathological outcome can be modified by rendering one of the melanoma lesions avascular. This "devitalisation" procedure essentially consists of ligaturing a tumour so that it is devoid of arterial blood supply. Crucially, the "devitalised" tumour tissue has to be left *in situ* for the effect to be observed. Thus, the evidence from this model seems to imply that loss of blood supply enables the mounting of an immune response by the animal to the tumour cells. Since the similar devitalisation treatment of normal tissue evokes no similar immune response there is evidence that the tumour cells carry "abnormal" antigens. It is possible that devitalisation results in release of antigens that are normally inaccessible, but were this the case there could be no general response to the evoked immunological reaction; i.e., the antigens recognised by the cytotoxic T-cells must be exhibited by the tumours even under normal metabolic conditions. It is necessary to conclude, therefore, that the failure of immune surveillance under aerobic conditions is due to diminished or absent antigen recognition. One possible explanation for this may be that the aerobic metabolism of malignant cells generates products that inhibit the antigen recognition process, as postulated.

Conclusion

In this paper I have outlined some general properties of cancer and drawn attention to the importance of the acquisition of the transmigratory ability by malignant cells. The specific factors responsible for this cardinal property may differ in different tumours but are likely to be associated with the expression of neoantigens. Thus, to escape the consequences of immune surveillance by the host, malignant cells must also possess a means of moderating or eliminating antigen recognition. It is proposed that this is connected with the deranged oxidative metabolism of malignant neoplasms, the products of which inhibit the normal functioning of antigen-presenting cells.

If the general argument set out herein is correct, two consequences of practical significance follow: (1) agents that enhance oxidative stress in the locality of pre-malignant or malignant cells will act as co-carcinogens and should be avoided or abrogated; and (2) alternative means of neoplastic antigen presentation, that separate this process from juxtaposition to the metabolic products of malignant tissue, should prove more efficient at inducing an immune response by the host. The reported therapeutic successes obtained from the use of tumour vaccines lend some support to these considerations.

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