DNA Release and Uptake Associated with the Development of Pleomorphic Cells in Mammalian Skin Autotransplants

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ABSTRACT

Objective: *Although several in vitro studies have demonstrated active release of DNA by living cells, there is still doubt. There are no such in vivo studies (1). The following experiment is an in vivo study to determine whether DNA release and uptake by cells and tissues occur and can be related to normal growth and differentiation, abnormal growth and cancer.*

Methods: *Epidermal and full-thickness ear-skin grafts were separately autotransplanted into two groups of mice. In a second group, host mice were labelled with tritiated thymidine and autografted separately, with unlabelled epidermal and full-thickness ear-skin grafts. Animals were sacrificed regularly in both cases.*

Results: *Full thickness grafts revealed cysts in 15 out of 16 grafts, with well-differentiated squamous epidermis, DNA labelling of dermal fibroblasts and no DNA labelling of epidermal cells. Epidermal grafts revealed cysts in six out of 20 grafts, with epidermal cells variable in shape and arrangement; some appeared normal but others were two to four times larger, forming solid nests of cells. In some grafts, there were spindle-shaped pleomorphic cells loosely interconnected. DNA labelling was observed in occasional epidermal cell. Two lung adenocarcinomas were found.*

Conclusion: *These results suggest active release of DNA by host cells and DNA uptake by grafted cells. This phenomenon and the differential uptake of DNA labelling of epidermal and dermal cells in the epidermal and full-thickness grafts suggest an association with abnormal, even pleomorphic epidermal cell behaviour due to the interference of dermal/epidermal interacting factors.*

Keywords: Cancer, cellular and genetic factors, growth and differentiation, intercellular exchange

Liberación y Absorción de ADN Asociada con el Desarrollo de Células Pleomórficas en Autotrasplantes de Piel en Mamíferos

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RESUMEN

Objetivo: *Aunque varios estudios* in vitro *han demostrado la liberación activa de DNA por las células vivas, todavía persisten las dudas. No existen tales estudios* in vivo *(1). El siguiente experimento constituye un estudio* in vivo *para determinar si hay liberación y absorción de ADN por parte de las células y los tejidos, y si estos procesos guardan relación con el crecimiento normal y la diferenciación, así como con el crecimiento anormal y el cáncer.*

Métodos: *Injertos de piel de la oreja, tanto de espesor total como epidérmicos fueron autotrasplantados por separado a dos grupos de ratones. En el segundo grupo, ratones huéspedes fueron etiquetados con timidina tritiada, y autoinjertados, por separado, con injertos de piel de la oreja no etiquetados, tanto de espesor total como epidérmicos. En ambos casos, fue necesario sacrificar animales de manera regular.*

Resultados: *Los injertos de espesor total revelaron quistes en 15 de cada 16 injertos, con epidermis escamosa bien diferenciada, etiquetado ADN de fibroblastos dérmicos, y no etiquetado ADN de células epidérmicas. Los injertos epidérmicos revelaron quistes en seis de 20 injertos, siendo las células epidérmicas variables en forma y ordenamiento. Algunas parecían normales, pero otras eran de dos a*

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cuatro veces mayores, y formaban anidamientos celulares sólidos. En algunos de los injertos, se presentaron células pleomórficas en forma de huso, interconectadas con laxitud. Se observó etiquetado de DNA en células epidérmicas ocasionalmente. Se hallaron dos adenocarcinomas pulmonares.
Conclusión: *Estos resultados sugieren la liberación activa de ADN por las células huéspedes y la absorción de ADN por las células injertadas. Este fenómeno y la absorción diferencial de etiquetado de ADN de células dérmicas y epidérmicas en los injertos epidérmicos y de espesor total, sugieren una asociación con el comportamiento celular anormal, e incluso pleomórfico epidérmico, debido a la interferencia de los factores dérmicos/epidérmicos interactuantes.*

Palabras claves: Cáncer, factores genéticos y celulares, crecimiento y diferenciación, intercambio celular

West Indian Med J 2011; 60 (3): 258

INTRODUCTION

Recently, there has been increased interest in two areas of research:

* the role of intercellular and inter-tissue factors in normal growth and differentiation and abnormal neoplastic growth (2, 3).
* the role of one category of these factors, the extracellular and circulating genetic material (RNA/DNA) in the homeostatic control of growth, differentiation, cancer development and spread (4).

This paper presents historical data and current research (5−10) on these research areas and attempts to link some of the current ideas on cancer development and spread into one unifying concept.

In embryological tissues, Grobstein (11) noted the importance of epithelial mesenchymal interaction for the development of the sub-mandibular gland and kidney, Golosow noted the same (12) for the pancreas, and Auerbach (13) for the thymus in mice.

In adult tissues, studies of feather development showed that tract specificity of the feather papilla was a function of the epidermal cells of the papilla, and not the dermis (14) while studies in man (15), guinea pigs and hamsters (16) found that the dermis determined epidermal differentiation. Thus, it was not clear whether the well-differentiated state of adult tissues was dependent on a continuous interchange of cellular factors for the maintenance of its state of differentiation.

Today, viruses are regarded as causative agents in some tumours *viz* HTLV-1, endemic in the Caribbean, is responsible for lymphoma/leukaemia. In the late 1960s this was not so. Viruses were not thought to be carcinogenic, although transmissible agents were detected in chemically induced sarcoma and radiation induced lymphoma. Kaplan (17) stated that "if exogenous chemical or physical agents produce leukaemia, viruses could be the universal common denominator in the aetiology of murine leukaemia", implying that every cell has a latent virus which can be activated by a carcinogenic agent. More plausible is the speculation that every cell has its own 'virus-like' mobile genetic elements which form part of its own exchange growth control mechanism, as the active principle in the virus is its nucleic acid – RNA/DNA.

Di Mayorca (18) has shown that DNA extracted from tissue culture cells infected with polyoma virus was infective. Ito (19) has found that Shope papilloma virus was carcinogenic. Chen (5) also noted that earlier research has shown that nucleic acid extracted from animal tumours and human tumours grown *in vitro* was tumourigenic in 9.9% of 246 injected newborn mice.

These studies show that mobile nucleic acids can affect growth and differentiation, which raises the question whether cellular exchange of genetic material is a naturally occurring phenomenon. This idea seems more plausible by Oppenheimer's work (20) which showed that implantation of inert plastic film in rat resulted in sarcoma formation, while perforated film reduced the incidence.

A series of experiments were subsequently designed to assess the following:

* whether intercellular and inter-tissue factors were important for growth differentiation and abnormal neoplastic growth.
* to determine if genetic material, in particular DNA, was one of those factors.

SUBJECTS AND METHOD

Full thickness ear-skin graft was obtained from 16 Balb 3 – 4-month old white mice; epidermal ear-skin graft was obtained from 20 Balb 3 – 4-month old mice. Full thickness and epidermal grafts were individually and separately autotransplanted intraperitoneally. Animals were sacrificed weekly from the 5th day to the 28th day and at the 70th day and 165th day. Grafts and graft sites were biopsied, sectioned and stained with haemotoxylin and eosin (H+E).

To determine whether one of the intercellular factors was DNA, 12 Balb white mice, 3 – 4 months old, were divided into 3 groups of 4. One group served as control. The second group was used for autotransplantation of full thickness skin graft. The third group was used for autotransplantation of epidermal skin grafts. The right ear of each Balb mouse was excised and used to prepare either full thickness or epidermal skin grafts and was stored at 4°C awaiting auto-intraperitoneal transplantation. Each earless

mouse was injected with tritiated thymidine and autotransplanted with either full thickness skin or epidermal skin graft at least 30 hours after tritiated thymidine injection. Animals were sacrificed at 10, 24, 48 and 80 hours. Grafts and graft sites were biopsied, sectioned, stained with H+E and developed autoradiographically. Cells with ten or more labelling grains were counted.

RESULTS

In the full-thickness skin transplants, there were distinct cysts in 15 out of 16 grafts, lined with well-differentiated squamous keratin, producing epidermis and surviving at least 165 days (Fig. 1.3). Hair follicles and sebaceous glands were few. Cartilage was present occasionally. No tumours were

Fig 1.1: Photomicrograph of full thickness ear-skin before autotransplantation. Low power x 100.

Fig. 1.2: Photomicrographs of pure epidermal graft before transplantation. High power x 400.

Fig. 1.3: Photomicrographs of full thickness ear-skin after autotransplantation into peritoneum for 7 days. The epidermis is well-differentiated. Keratin is present. High power x 400.

detected in the animals. In contrast, in the pure epidermal skin transplant, there were 6 cysts out of 20 grafts: 1 completely and 5 incompletely lined with well-differentiated squamous keratin producing cells, varying in size from normal to 2−4 times larger. Survival time of grafts was less than 165 days. Few hair follicles were present and arranged

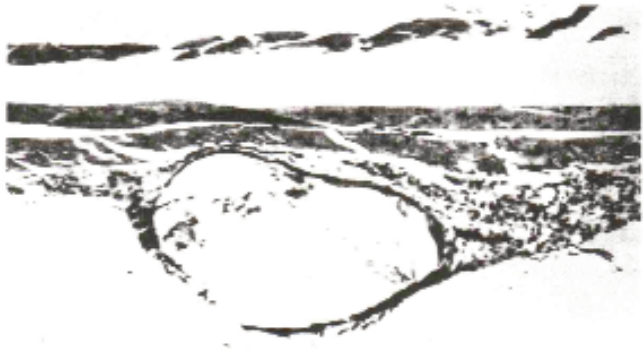

Fig. 1.4: Photomicrographs of cyst formed from pure epidermal graft autotransplanted for 21 days. Well-differentiated epidermis is present. No sebaceous gland or hair follicles present. Lower power x 100.

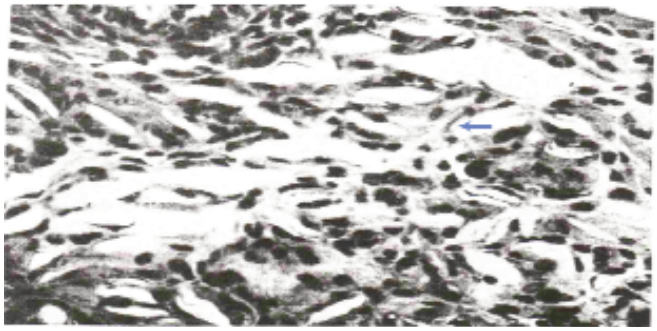

Fig. 1.5: Photomicrographs of pure epidermal graft autotransplanted for 21 days showing netted arrangement for pleomorphic epidermal cells loosely interconnected, pointed by arrow. Keratinous flakes present within microspaces. High power x 400.

in abnormal pattern with solid nests of cells present. No sebaceous glands nor cartilage cells were seen. Two papillary pulmonary adenocarcinomas were seen in epidermal autotransplant animals (Fig. 1.6).

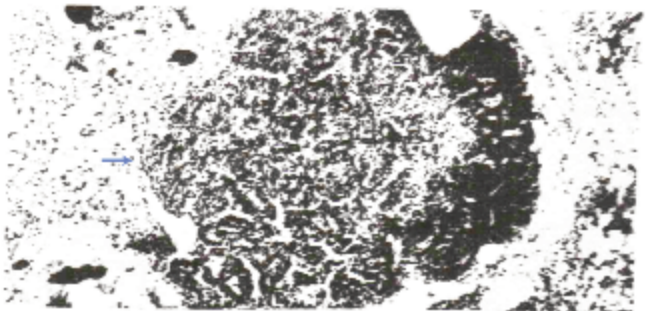

Fig. 1.6: Photomicrographs of pulmonary papillary adenocarcinoma found in mouse autotransplanted with pure epidermal graft for 165 days. Low power x 100.

In four transplants, between 10−28 days old, there were abnormal pleomorphic cells. These varied from flattened

spindle shaped, to rectangular looking cells, loosely interconnected leaving microspaces between neighbouring cells, giving a netted appearance and containing in some areas, flakes of keratin (Fig. 1.5). There were no inflammatory cells in these sections.

Survival of grafts cells was indicated by staining of the nuclei and the hyperplasia of the epidermis. After 10 hours, there was a slight outpouring of inflammatory cells composed mainly of lymphocytes, mononuclear cells and some polymorphs. None of the grafts cells was labelled at this stage.

In the 24-hour, 48-hour and 80-hour old grafts, the inflammatory responses were more acute and labelled lymphocytes and mononuclear cells were seen invading and mingling with the cells in the dermis of the full thickness skin grafts. In the 24-hour old epidermal graft, there were still few lymphocyes seen and none of these cells appeared dead or dying as revealed by pyknosis.

Labelling of cells was observed in both the epidermal (Fig. 1.7) and full thickness skin grafts. In the full thickness

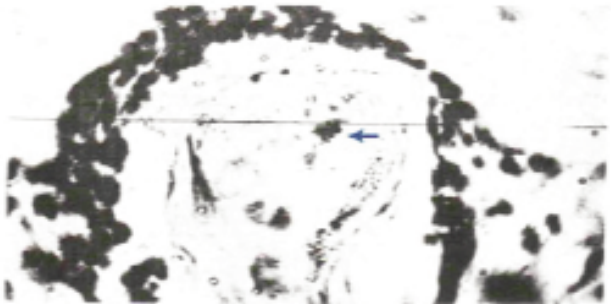

Fig. 1.7: Photomicrographs of autoradiograft of pure epidermal graft auto transplanted for 24 hours. Epidermal cells are surrounded by inflammatory exudates. Arrow points to labelled epidermal cell present. High power x 400.

skin grafts, however, labelling was noted only in the dermis (Fig. 1.8).

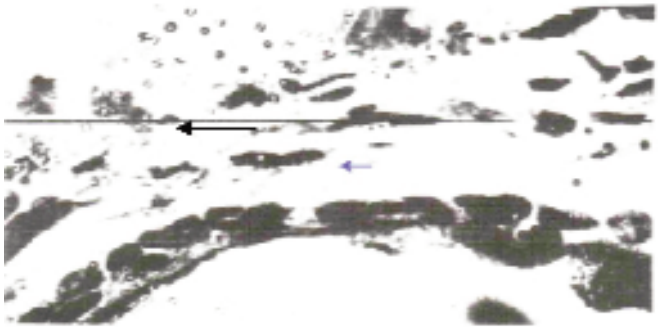

Fig. 1.8: Photomicrograph of autoradiograft of full thickness graft autotransplanted for 18 hours. Arrow points to labelled fibroblast present. High power x 400.

Table 1: The results of ear-skin autografts

Full-thickness ear-skin autografts				Epidermal ear-skin autografts	
Time (in hours)	No. of cells labelled per 100			No. of cells labelled per 100	
	Epidermis	Dermis	Host Lymphocytes	Epidermis	Host Lymphocytes
10	0	0	1	0	5
24	0	1	3	1	10
48	0	10	10	1	6
80	0	6	6	0	0

DISCUSSION

The results show that the epidermis, when transplanted with its underlying dermis, in the form of a full thickness skin graft, differentiates normally, assuming a squamous pattern, forming keratin, occasional hair follicles and sebaceous glands. The presence of cartilage cells warrants further study to determine the cartilage inductive factors in ear dermis, compared to non-cartilage inducing dermis, such as in the abdominal wall.

The histological differences between the epidermal and full thickness autografts suggest the role of intercellular and inter-tissue factors for normal growth and differentiation. The development of abnormal pleomorphic epidermal cells occurring in the absence of the dermal influences indicate the extent of dermal control in normal tissues. The development of pleomorphic changes in epidermal autografts by simply interfering with normal inter-tissue interaction without the need of external stimuli requires further investigation. Perhaps, this is the first description of such a phenomenon.

Support for this concept comes from Hayward Rosen and Cunha (21) who proposed that “disruption of homeostatic inter-tissue control mechanisms is responsible for the development of prostate cancer”. More recently, it has been shown that malfunctioning of signalling pathways between stromal fibroblasts and myoepithelial cells in breast cancer leads to invasion of epithelial cells (22).

Labelling of graft cells in the epidermis and dermis raises the question of the source and the nature of the labelling substance. It is unlikely that persistent circulating tritiated thymidine injected 30 hours previously was utilized, as the 10-hour old grafts were not labelled and it has been shown that the availability of tritiated thymidine is less than 24 hours after injection (23, 24).

As regards the source of the labelling material, cell death of host cells, including inflammatory cells could have released DNA breakdown products. But no dead or dying host cells were observed. The fact that epidermal cells of epidermal graft took up labelling, whilst epidermal cells of full thickness grafts did not, but the dermal fibroblasts did, indicates an active process and the dermis exerts some controlling influence in the process and the activity of the epidermis. Thus the active release by host cells and uptake of nuclear material by grafted cells remains a possibility. Stroun (25) in frog, Adam and McIntosh (26) in chicken and

Anker (27) in human lymphocytes found similar DNA active release by living cells.

The significance of this release of nuclear material by living cells and uptake by others is not clear. The fact that epidermal growth and differentiation is dependent on inter-tissue factors already discussed suggests that an important role of this exchange of nuclear material between living cells *in vivo* is to provide a basis for informational exchange in the normal control of growth and differentiation.

The labelling of the lymphocytes and other inflammatory cells was important to indicate labelling of the host. Migration of host lymphocytes and other inflammatory cells to the graft may suggest an active role of these cells in the DNA donor/recipient exchange mechanism. The fewer labelled cells at 80 hours, as compared to 40 hours, need further studies for clarification. Individual variation of response in a homeostatic dynamic system may be an explanation.

The DNA labelling of graft stromal fibroblasts is most likely due to release of DNA by host cells and uptake of DNA by the graft fibroblasts. This experiment done by Chen (5 − 7) was the first demonstration of active DNA release of living cells and DNA uptake by living cells in an *in vivo* auto-transplant mammalian system. The differential uptake of DNA by epidermal graft cells, the non-uptake by epidermal cells and uptake by stromal cells of full thickness grafts suggest some association with pleomorphic growth and neoplasia.

The finding of a high incidence of pulmonary adenocarcinomas in 2 out of 20 mice, 3 − 4 months old, in the epidermal grafted mice supports this. A recent study of Balb-nu mice reported that tumours were hardly detectable in mice up to the age of 22 months (28).

Chen (5) hypothesized that a system of exchange of intercellular and inter-tissue factors including genetic material (DNA/RNA) between cells and tissues exist. This is important for the homeostatic control of growth and differentiation, and as a corollary to
this hypothesis, any disturbance of the exchange system or the messages themselves result in abnormal growth and cancer.

This concept is supported by recent work of Gahan (29) who proposed that a DNA fraction can act as a messenger between cells and tissues. Chen (30) also hypothesized that DNA is released by living cancer cells and can transform adjacent and remote cells to cancer while Garcia-Olmo (4) proposed that a fraction of circulating DNA is responsible for cancer metastases.

To further understand intercellular and inter-tissue relationships, the author, working with collaborators on prostate cancer, has developed the first successful human prostate cancer mouse xenograft model (10), the first pair of metastatic and non-metastatic prostate cancer tumour line, derived from the same original tumour (9). Dong *et al* (31) have also identified the Asapi gene associated with prostate cancer metastasis in this metastatic tumour line. This xenograft human prostate cancer model can be used to determine if there is an exchange of micro RNAs and "micro DNAs" between cells and tissues. Identification of such mobile genetic elements will help in the understanding of normal and abnormal growth processes, of carcinogenesis, the mechanism of metastatic spread and in providing markers for cancer diagnosis, targets for therapy, predictors of the metastatic potential of early cancer and in the monitoring of patients on therapy.

ACKNOWLEDGEMENTS

The author wishes to thank Professor S Lavelle, Department of Experimental Medicine, University College, Galway, Ireland for his guidance and encouragement. Thanks are also due to Miss M Kavanagh and Miss C McNamara for their help in the preparation of histological sections and to Mr M Kelly for assisting with photographic reproduction.

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