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Correlation of donor age and telomerase activity with in vitro cell growth and replicative potential for dermal fibroblasts and keratinocytes

M.H. Ng [a,b], B.S. Aminuddin [c], S. Hamizah [b], C. Lynette [b], A.L. Mazlyzam [a,b], B.H.I. Ruszymah [a,b,*]

[a] *Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (National University of Malaysia), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia*
[b] *Tissue Engineering Centre, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia*
[c] *Ear Nose and Throat Consultant Clinic, Ampang Puteri Specialist Hospital, Selangor, Malaysia*

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Abstract Previous studies suggested telomerase activity as a determinant of cell replicative capacity by delaying cell senescence. This study aimed to evaluate the feasibility of adopting telomerase activity as a selection criterion for in vitro expanded skin cells before autologous transplantation. Fibroblasts and keratinoctyes were derived from the same consenting patients aged 9–69 years, and cultured separately in serum-supplemented and serum-free media, respectively. Telomerase activity of fresh and cultured cells were measured and correlated with cell growth rate, donor age and passage number. The results showed that telomerase activity and cell growth were independent of donor age for both cell types. Telomerase was expressed in freshly digested epidermis and dermis and continued expressing in vitro. Keratinocytes consistently showed 3–12 folds greater telomerase activity than fibroblast both in vivo and in vitro. Conversely, growth rate for fibroblast exceeded that of keratinocyte. Telomerase activity decreased markedly at Passage 6 for keratinocytes and ceased by Passage 3 for fibroblasts. The decrease or cessation of telomerase activity coincided with senescence for keratinocyte but not for fibroblast, implying a telomerase-regulated cell senescence for the former and hence a predictor of replicative capacity for this cell type. Relative telomerase activity for fibroblasts from the younger age group was significantly higher than that from the older age group; 69.7% higher for fresh isolates and 31.1% higher at P0 ($p < 0.05$). No detectable telomerase activity was to be found at later subcultures

* Corresponding author. Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (National University of Malaysia), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia. Tel.: +603 92897224, 91456067; fax: +603 26939687.
E-mail address: ruszy@medic.ukm.my (B.H.I. Ruszymah).

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for both age groups. Similarly for keratinocytes, telomerase activity in the younger age group was significantly higher ($p < 0.05$) compared to that in the older age group; 507.7% at P0, 36.8% at P3 and the difference was no longer significant at P6. In conclusion, the study provided evidence that telomerase sustained the proliferation of keratinocytes but not fibroblasts. Telomerase activity is an important criterion for continued survival and replication of keratinocytes, hence its positive detection before transplantation is desirable. Inferring from our results, the use of keratinocytes from Passage 3 or lesser for construction of skin substitute or cell-based therapy is recommended owing to their sustained telomerase expression.

Introduction

The use of autologous skin for grafting has a long history and can be traced since two thousand years ago in India [1]. The use of skin graft is necessary if not vital to accelerate wound closure and healing in burns and large open wound due to large skin tumour resection and traumatic injury. The major setbacks in skin grafting are limited donor sites and donor site morbidity. Repeated harvesting of available donor sites might be necessary for chronic ulcers or large burn areas and this is accompanied by lengthy hospital stays and high medical cost.

The constant need for skin grafts has led to many skin substitutes being available commercially. Advances in skin tissue engineering can now offer a laboratory fabricated autologous living skin substitute to patients. The synthesis of tissue engineered human skin in vitro requires extensive culture expansion of skin cells. The transplanted autologous skin substitute consisted of cells that are ideally 'young' and actively dividing will provide an immediate barrier to the environment and accelerate wound closure by the release of trophic factors of the transplanted cells [2]. It is a well accepted to date that all normal somatic cells have limited life span or replicative potential. Extensive culture expansion in vitro during the tissue engineering process will hence inevitably be associated with cellular aging.

Numerous studies have linked cell senescence to telomere shortening [3–6]. Telomere shortening, a physiological condition well documented is due to the incomplete replication at the 3′ termini of each chromosome thus resulting a loss of 50–60 bp of the chromosome ends (telomere) with each round of cell replication [4]. As a result, cell senescence or apoptosis sets in when the telomere length becomes too short. However, the nature's way of overcoming this problem is by activating telomerase, a special ribonucleoprotein reverse transcriptase that adds telomeric sequences to the ends of chromosomes. Actively dividing cells for example the basal cells of the epidermis, bone marrow cells, endometrium cells and crypt cells in the small intestine, reproductive cells and cancer cells express telomerase in order to counter the rapid telomere attrition due to their exhaustive proliferation [7]. Among these telomerase-expressing cells, keratinocytes, hematopoietic and mesenchymal stem cells have been explored for cell transplantation. Despite their telomerase positivity, these cells have limited replication capacity. Studies have shown that telomerase activity in these cells decreased with sequential subculturing and hence a function of cell replication capacity in vitro [8].

In our previous study, we found an age-dependent decrease of telomerase activity in bone marrow derived mesenchymal stem cells (MSCs) with donor age [9]. This decrease in telomerase activity has been implicated with the observed decline in cell growth rate of MSCs from donors above 40 years old [10].

In line with our interest to develop tissue engineered skin models, we are keen to find a marker that could help predict the replicative potential of the cells that are to be transplanted. We embarked on the study with the assumption that cells with high telomerase activity would possess continued replicative capacity upon transplantation and engraftment and hence, theoretically, make good candidates for autologous skin reconstruction The specific objectives of the study was to establish possible correlation of telomerase with donor age, cell growth rates, capacity of keratinocytes and fibroblasts to divide. Based on our previous observation of the dramatic decline in telomerase activity for donors above 40 years old, two age groups will be investigated in this study; above 40 years and below 40 years.

Materials and methods

Cell culture in vitro

The study was approved by the Institutional Ethics Committee (UKM ref no.) Skin samples were obtained from biopsies of patients undergoing