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Phenotypic and functional modulation of 20–30 year old dermal fibroblasts by mid- and late-gestational keratinocytes in vitro

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ABSTRACT

Fetal wound healing occurs rapidly and without scar formation early in gestation, but the mechanisms underlying this scarless healing are poorly understood. This study explores the phenotypic and functional modulation of 20–30 year old dermal fibroblasts by mid- and late-gestational keratinocytes (KCs) in vitro. Human KCs of different gestational ages were isolated, characterized, and co-cultured with human 20–30 year old fibroblasts. Gene expression and protein levels of TGF-β family members, precollagen, collagen, matrix metalloproteinases (MMPs), and the tissue inhibitors of metalloproteinases (TIMPs) were measured in the fibroblasts. Mid-gestational KCs promoted faster proliferation and migration of fibroblasts than late-gestational KCs. Additionally, significant differences in gene expression and protein levels of some markers were observed in fibroblasts co-cultured with mid- or late-gestational KCs. Fibroblasts co-cultured with mid-gestational KCs for 48 h exhibited downregulated gene expression of precollagen 1, collagen 1, TGF-β1, TGF-β2, TIMP-2 and TIMP-3, while precollagen 3, collagen 3, TGF-β3, and MMP-1, -2, -3, -9 and -14 were upregulated. In contrast, late-gestational KCs exhibited downregulated TIMP-1, TIMP-2 and TIMP-3 levels, while collagen 1, TGF-β2, TGF-β3, and MMP-2, -3, -9 and -14 were upregulated. Moreover, statistically significant differences in expression levels of precollagen 1, precollagen 3, collagen 1, TGF-β1, -β2, and -β3, MMP-1, -3 and MMP-14, TIMP-1 and TIMP-2 were found between fibroblasts co-cultured with mid- and late-gestational KCs. Furthermore, cytokine levels of IL-1a and HB-EGF were found to be statistically different between conditioned medium from mid- and late-gestational KCs. Therefore, the gestational age of KCs appears to have an important effect on scarless wound healing in the human fetus.

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1. Introduction

The primary function of the skin is to serve as a protective barrier against the environment. Loss of integrity of large portions of the skin as a result of injury or illness may lead to major disability or even death. Therefore, the primary goals of wound treatment are rapid wound closure and a functional and esthetically satisfactory scar.

It is well documented that fetuses and adults follow different regimens for skin wound repair [1]. In adults, wound healing is characterized by intense inflammation and scar formation. In contrast, wound healing during the first two trimesters of gestation involves a diminished inflammatory response, decreased angiogenesis, and the absence of contraction and scar formation. The behavioral patterns of fetal skin cells, including epidermal keratinocytes (KCs) and dermal fibroblasts, are important for the healing process, however, the detailed mechanisms of scarless wound healing in the fetus are poorly understood.

Currently, wound healing is thought to be mediated by human dermal fibroblasts. These are the primary cells responsible for reconstructing the connective tissue in the process of fetal and postnatal wound healing, and are involved in important regulatory events during wound healing [2–4]. Therefore, one of the hallmarks of skin wound healing is the proliferation and migration of fibroblasts [5]. Epidermal KCs secrete soluble factors to induce dermal fibroblast proliferation and migration that enhances wound repair through regulation of the production or activity of TGF-β, precollagen, collagen, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs) [6,7]. However, the effect of prenatal KCs on postnatal fibroblast wound healing behavior remains unclear.

In present study, we used a unique model system to elucidate the effects of human mid- and late-gestational KCs on the phenotype and function of 20–30 year old dermal fibroblasts and determined which factors and mechanisms of prenatal wound healing could be applied to postnatal wound repair. Cellular proliferation and migration were examined, and the secretion of TGF-β, precollagen, collagen, MMPs and TIMPs in fibroblasts was measured, as these markers are involved in scarless wound healing. Furthermore, the levels of several important cytokines involved in wound healing were measured in conditioned medium from fetal KCs.

2. Materials and methods

2.1. Cell culture

Skin samples used for primary KCs and fibroblasts are listed in Table 1. Women undergoing termination of pregnancy due to accidental trauma, such as car crashes or burns, gave written informed consent for the use of fetal tissue for this research. Fetal skin specimens from the lower leg were divided into two groups: mid-gestation (gestational age 20–23 weeks, 3 males and 2 females) and late-gestation (gestational age 29–34 weeks, 3 males and 2 females). In addition, fibroblasts were obtained from the dermis of donors undergoing surgical debridement (aged 20–30 years, 3 males and 2 females). KCs were obtained from all three groups. Skin samples used for the experiments were obtained according to the ethical rules for human experimentation stated in the 1975 Declaration of Helsinki under approval from the Shengjing Hospital of China medical university ethics committee. Primary cultures of KCs and fibroblasts were prepared as described previously [8,9]. Briefly, full-thickness skin samples were incubated at 4 °C overnight in Dispase II (Roche Applied Science, Indianapolis, IN, USA), followed by removal of dermal components via collagenase digestion. After mincing of dermal components, isolated fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA, USA). Following digestion, primary KCs were released from the epidermis by 0.25% trypsin digestion and cultures were initiated using tissue culture flasks coated with collagen (Becton Dickinson Labware, Bedford, MA, USA), in Epilife™ growth medium (Invitrogen Ltd, Paisley, UK) supplemented with 1% human keratinocyte growth supplement (Invitrogen Ltd, Paisley, UK). After the fifth passage, these cultures and co-cultures of these cells with human 20–30 year old fibroblasts were used for further analysis and characterization.

Table 1 – Profiles of KCs and fibroblasts used in the study.

Case	Gestation age (weeks/years)	Sex	Race	Location of skin
1	20 w	Male	Chinese	Lower leg
2	23 w	Male	Chinese	Lower leg
3	23 w	Male	Chinese	Lower leg
4	21 w	Female	Chinese	Lower leg
5	23 w	Female	Chinese	Lower leg
6	29 w	Male	Chinese	Lower leg
7	30 w	Male	Chinese	Lower leg
8	34 w	Male	Chinese	Lower leg
9	29 w	Female	Chinese	Lower leg
10	30 w	Female	Chinese	Lower leg
11	20 y	Male	Chinese	Lower leg
12	24 y	Male	Chinese	Lower leg
13	25 y	Male	Chinese	Lower leg
14	26 y	Female	Chinese	Lower leg
15	30 y	Female	Chinese	Lower leg

2.2. Co-culture of 20–30 year old fibroblasts with KCs

For co-culture of 20–30 year old fibroblasts with KCs, KCs were first seeded at a density of 10^5 cells/cm^2 on transwell clear polyester membrane inserts with a 0.4 mm pore size (Corning Costar, Tewksbury, MA, USA) in serum-free low calcium keratinocyte growth media and grown until confluent. The medium was then changed to high-glucose DMEM with 10% FBS and the cells were raised to an air-liquid interface. Prior to co-culture, fibroblasts were seeded onto six-well plates in high-glucose DMEM with 10% FBS for 48 h, and cultured in serum-free medium for an additional 24 h. After washing with phosphate-buffered saline (PBS), the KC membranes were transferred onto the cultured fibroblasts and maintained in serum-free low calcium keratinocyte growth medium supplemented with 5 ng/ml EGF for two days.