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Spatial and temporal age-related spectral alterations in benign human breast tissue

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ABSTRACT

Epidemiological evidence suggests that cancers attributable to exogenous carcinogenic agents may appear decades after initiating exposures. Environmental factors including lifestyle and/or diet have been implicated in the aetiology of breast cancer. Breast tissue undergoes continuous molecular and morphological changes from the time of thelarche to menopause and thereafter. These alterations are both cyclical and longitudinal, and can be influenced by several environmental factors including exposure to oestrogens. Research into the latent period leading to breast carcinogenesis has been mostly limited to when hyperplastic lesions are present. Investigations to identify a biomarker of commitment to disease in normal breast tissue are hindered by the molecular and histological diversity of disease-free breast tissue. Benign tissue from reduction mammoplasties provides an opportunity to study biochemical differences between women of similar ages as well as alterations with advancing age. Herein, synchrotron radiation-based Fourier-transform infrared (SR-FTIR) microspectroscopy was used to examine the terminal ductal lobular epithelium (TDLU) and, intra- and inter-lobular epithelium to identify spatial and temporal changes within these areas. Principal component analysis (PCA) followed by linear discriminant analysis of mid-infrared spectra revealed unambiguous inter-individual as well as age-related differences in each histological compartment interrogated. Moreover, exploratory PCA of luminal and myoepithelial cells within the TDLU indicated the presence of specific cells, potentially stem cells. Understanding alterations within benign tissue may assist in the identification of alterations in latent pre-clinical stages of breast cancer.

1. Introduction

The clinical manifestation of breast cancer is the final expression of a complex sequential process that begins with exposure to a causative agent [1]. Tumour formation involves temporal alterations in genetic morphology or expression, which directly or indirectly disturbs normal cellular regulation of proliferation and growth inhibition, leading to malignancy [2]. The period from an initiating event to tumour formation is termed the “latency period” [3]. This definition implies that cancers in which environmental exposures play a role arise several years after initiating exposures [1,4]. This latency period may be of different lengths depending on the type, timing and length of exposure as well as inherent predisposition to the particular type of cancer [5].

Exposure to carcinogens will certainly vary significantly between individuals as will their response to such agents [6,7]. Factors that predispose women to a risk of breast cancer include early menarche, late menopause, nulliparity or delayed parity and, use of contraception or hormone replacement therapy [8,9]. Such characteristics are associated with increased oestrogen exposure. It is now accepted that “Westernized” lifestyle either through immigration or adoption of Western diet are likely causative factors for breast or other hormone-dependent cancers [1,10].

Little is known regarding the molecular changes that may develop before the appearance of pre-clinical or clinical breast cancer [11]. Changes that appear at the initiation stage or during the latency period may provide useful biomarkers for early identification of at-risk women. Also, these changes may be temporary, regressive, permanent or progressive [11,12]. Biomarkers that could identify lesions with a low risk of progression towards malignancy or even the chance of regression would be advantageous [13–15].

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Women exhibiting high-risk alterations might be encouraged to make appropriate lifestyle alterations to “rectify” such changes [16].

To facilitate understanding of pathological processes involved in breast carcinogenesis, we first need to identify physiological differences within tissues from similarly-aged women as well as age-related alterations. The areas wherein these variations are most likely to occur are within the terminal ductal lobular unit (TDLU) along with the supporting intra- and inter-lobular stroma (Fig. 1a). These areas are thought to be responsible for cancer initiation processes [11,17,18]. The TDLU consists of terminal ductules ending in acini, bounded by luminal epithelial cells, which are surrounded by myoepithelial cells (Fig. 1b). TDLUs have different compositions depending on their developmental stage from pre-puberty to menopause (Fig. 1c). The pre-pubertal “simple” TDLU consists of one central ductule with three or four branches. After menarche, the TDLU's morphology depends on the stage of the menstrual cycle with luminal cells growing in size as the cycle progresses from the follicular to the luteal phase. During pregnancy or lactation, the TDLU hypertrophies and remains in a similar state to the luteal phase. Post-menopausally, the lobule has fewer ductules and a denser intralobular stroma. With advancing age, the TDLU undergoes complete atrophy but the branching duct tree remains. In cancer, the micro-architecture of the TDLU is disturbed.

Breast tissue from reduction mammoplasties provides an opportunity to study spatial and temporal variations that may exist within the TDLU and surrounding areas of the mammary gland when there is no evidence of malignant or pre-malignant changes. The cancer risk in this population is comparable or marginally reduced relative to the general population [19,20]. Biospectroscopy techniques can lend novel insights into structural alterations in target cells [21,22]. This approach has been employed to detect alterations associated with cancer in various tissues [23–27]. Its potential in identifying biomarkers that can be used in cancer screening has also been examined [28,29].

Synchrotron radiation-based Fourier-transform infrared (SR-FTIR) microspectroscopy is superior to conventional bench-top systems in that it gives enhanced spectral signal-to-noise ratio (SNR) with greater spatial resolution. The main reason is that a synchrotron source emits a collimated light beam that is more brilliant than that of a typical globar source found in a bench-top spectrometer. This provides an excellent SNR that is 1000 times greater to that of conventional IR sources and allows spatial resolutions as small as 10 μm [23,30].

Interrogation of biological tissues by IR spectroscopy can results in thousands of spectra, necessary due to the complex chemical composition of cells. Such a large amount of data obtained in an increasingly typical spectrochemical experiment may be analysed using multivariate analysis [31,32]. This allows data simplification for visual representation and exploratory analysis. Two of the commonly utilized multivariate analysis approaches are: principal component analysis (PCA) and linear discriminant analysis (LDA) or a combination of both [33]. This study aimed to identify spectral differences in breast tissue of women of similar ages as well as changes with increasing age. This could be a first step towards the recognition of the initiation/early promotion stages of breast cancer.

2. Materials and methods

2.1. Sample preparation

Human breast tissue was obtained from eleven patients who had undergone reduction mammoplasty for indications other than breast-related pathology. Consent was taken with ethical approval (Research Ethics Committee reference: 10/H0308/75) according to the Declaration of Helsinki. Five individuals were aged 20 to 29 y, three were aged 30 to 39 y and three were aged 40 to 49 y. Breast

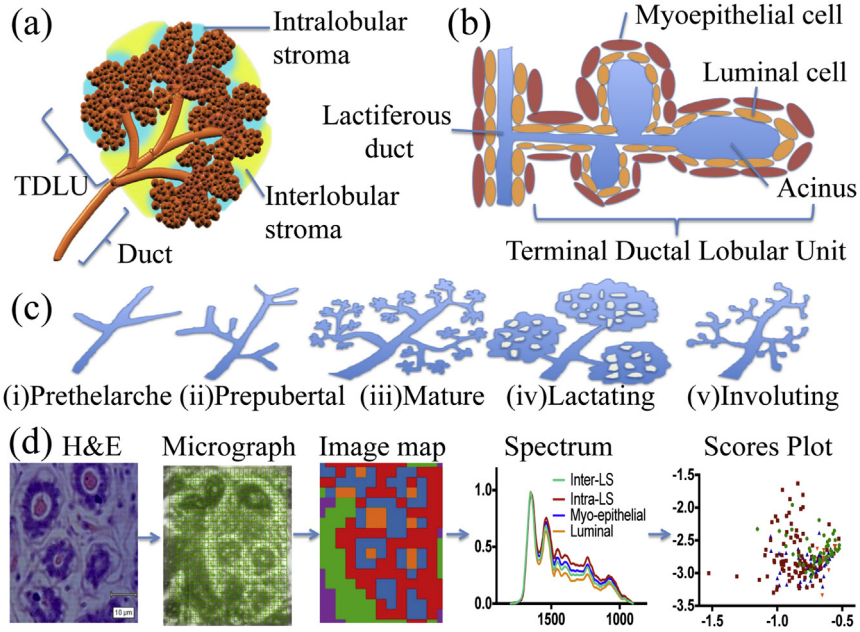

Fig. 1. Study overview. (a) Diagram representing a lactiferous duct with an aggregation of TDLUs. The space between acini within the TDLU is occupied by intra-lobular stroma while the space between different TDLUs is occupied by inter-lobular stroma. (b) Diagrammatic interpretation of the terminal ductal lobular unit (TDLU), illustrating the types of cells that surround an acinus. (c) Diagrammatic description of the developmental progression of the TDLU with advancing age. (d) Example of principal component analysis and linear discriminant analysis (PCA-LDA) of TDLU, intra-lobular stroma and inter-lobular stroma. The tissue section was selected from a subject within the 40–49 y age group. (1) Parallel sections were stained with H&E for histological representation. (2) Numbered grid overlays were added to micrographs of the sections to aid spectral selection. (3) Image maps were produced from which spectra were extracted. (4) Class means representing spectral differences between different cell types. (5) PCA-LDA scores plots of different cells where each spectral point is derived from the average of 5 IR spectra.