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Review

Intracrine oestrogen production and action in breast cancer: An epigenetic focus

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ABSTRACT

Epigenome changes have been widely demonstrated to contribute to the initiation and progression of a vast array of cancers including breast cancer. The reversible process of many epigenetic modifications is thus an attractive feature for the development of novel therapeutic measures. In oestrogen receptor α (hereinafter referred to as ER) positive tumours, endocrine therapies have proven beneficial in patient care, particularly in postmenopausal women where two-thirds of tumours are oestrogen dependent. However, resistance to such therapies is a common feature amongst individuals. In the current review, we discuss the influence that epigenetics has on oestrogen dependent breast cancers, in particular (i) the production of intracrine oestrogen in postmenopausal women, (ii) the action of oestrogen on epigenetic processes, and (iii) the links between epigenetics and endocrine resistance and the current advancements in epigenetic therapy that target this process.

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Contents

1. Introduction

The majority of postmenopausal breast cancers are dependent on oestrogens, particularly the biologically potent 17β-estradiol (E2), for sustained growth and proliferation [1]. In premenopausal women, oestrogens arise from the ovary and behave in a classic endocrine manner through circulation, acting on target tissues that express specific oestrogen receptors [2]. Following menopause, oestrogen ceases to be produced by the ovaries, and circulating levels are reduced. Despite this, adrenal androgen substrates remain in abundance allowing for the conversion to oestrogens. In postmenopausal women this conversion occurs in peripheral tissues such as muscle, bone, skin and the brain [3–6]. In the breast, adipose tissue becomes the major source of local oestrogen production through the expression of metabolising enzymes and the peripheral conversion of androgens [4]. The transformation of this non-classical hormone producing site into an intracrine source of intra-tumoural oestrogen is a key feature of ER-positive breast carcinoma cells.

Our understanding of oestrogen biosynthesis and its actions in the breast tumour microenvironment has led to frontline endocrine therapies such as selective oestrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) that are used in the neo- and adjuvant setting [7]. In particular, AIs have become the gold standard for the treatment of postmenopausal women with oestrogen-dependent tumours [8]. One of the major challenges facing endocrine therapy is the development of resistance to prolonged oestrogen inhibition. Determining the cause or how this

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resistance can be alleviated in combination with other therapeutics is therefore an active focus of research. Changes in the epigenome are an underlying facet associated with all cancers studied to date, in particular breast cancer [9]. In this review, we describe current advances in our understanding of the epigenome changes associated with breast cancer that affect oestrogen biosynthesis, that are influenced by oestrogen and those that contribute resistance to endocrine therapy.

2. Overview of the dynamics of epigenetic regulation

Epigenetics describes a broad range of DNA and histone modifications that may modulate gene expression without altering the underlying coding sequence. This encompasses DNA methylation as well as histone modifications including acetylation, methylation, phosphorylation, sumoylation, ubiquitination and ADP ribosylation. In combination, these processes work to regulate gene expression, mediate X-chromosome inactivation and facilitate genomic imprinting.

DNA is organised within the cell nucleus as a packaged structure known as chromatin. This dynamic configuration consists of DNA strands wrapped around histone proteins, and is consistently changing according to the transcriptional and replication needs of the cell. Epigenetics is the means by which chromatin structure is configured to prevent or allow access of transcriptional machinery to target genes, thus controlling gene expression levels. When a genomic region is in a tightly packed chromatin structure, transcription is repressed. Conversely, loosely packed chromatin allows transcription of local genes to initiate [10]. DNA methylation and histone modifications work to determine the open or closed state of chromatin, and therefore exert a significant influence on gene expression.

DNA methylation is a phenomenon that occurs in both prokaryotes and eukaryotes [11,12]. In mammals, DNA methylation occurs only on cytosine residues that are immediately 5′ of a guanine, described as a CpG site [13]. This dinucleotide is under-represented in the mammalian genome, however they appear to occur in clusters referred to as CpG islands. CpG islands are defined as nucleotide stretches greater than 200 bp in length with a CG percentage of at least 60% [14], and occur in the promoter regions of approximately 50% of genes [15]. However, methylation of CpG sites outside of CpG islands and also within coding exons can effect gene expression. Hypermethylation of promoter CpG regions leads to a recruitment of histone modifying enzymes to the chromatin, resulting in acetylation of local histones and compaction of the chromatin structure to prevent access for transcriptional machinery [16]. Thus, an inverse correlation exists between promoter methylation levels and transcription levels, whereby hypermethylayion results in low gene expression and hypomethylayion results in high gene expression. Methylation of even a single CpG site within a gene regulatory region may also effect gene expression by interfering with the binding of transcription factors to their response elements [17].

Cytosine methylation is mediated by three distinct DNA methyltransferase enzymes: DNMT1, which maintains existing methylation patterns [18], and DNMT3a and DNMT3b, which establish *de novo* methylation of CpG sites during developmental stages [19] but also to a lesser degree participate in the maintenance of methylation patterns during DNA replication [20]. All three catalyse the transfer of methyl groups from S-adenosyl-L-methionine to the 5′ position of cytosines as part of a CpG dinucleotide [15]. A number of mutations of the DNMTs have been reported, and these are associated with colorectal cancer [21], acute myeloid leukaemia [22] and acute monocytic leukaemia [23]. Additionally, overexpression of the DNMTs often occurs in cancer and contributes to the hypermethylation often observed in tumour tissue [24]. More recently, mechanisms of DNA demethylation have come to light, with research suggesting both active and passive means of removing methyl groups. Passive demethylation can occur when there is an error in DNA methylation maintenance during replication, resulting in a loss of methylation on the newly synthesised daughter strand [25]. Active demethylation conversely, relies upon a range of enzymes that can hydroxylate, further oxidise or deaminate methylated cytosines independent of DNA replication. The current model of DNA demethylation involves a two-step process: hydroxylation of methylated cytosines by ten-eleven-translocation (TET) proteins [26], followed by deamination by activation-induced cytidine deaminase (AID) enzymes [27]. Alternatively, it has been proposed that following hydroxylation, cytosines may be further carboxylated and then subsequently entered in to the base excision repair pathway [28]. Another possible explanation for demethylation is that hydroxymethylated cytosines are not recognised by maintenance DNMTs, therefore TET protein-mediated hydroxylation of methylated cytosines would result in a loss of methylation during subsequent replication cycles [29]. Three TET family members have been reported to date, including TET1, TET2 and TET3 [25]. Each appears to have a distinct function within a variety of cellular contexts [30]. Mutations in TET2 have been reported in T-cell lymphoma [31] and acute myeloid leukaemia [32], whilst other TET family members are less well characterised.

DNA methylation often works in synergy with histone modifications to regulate gene expression. Hypermethylation of CpG sites leads to a recruitment of methyl-binding proteins, which in turn recruit histone deactylases (HDACs) to the region and repress gene transcription by closing chromatin structure [16,33]. Eighteen different HDAC genes have been identified to date subdivided in to two major subgroups [34], however all HDACs function to deacetylate not only lysine residues in histone tails but non-histone proteins including transcription factors [35]. The action of HDACs is counterbalanced by histone acetyltransferases which are able to introduce acetylation to histones, loosening their structure in order to become more accessible to transcriptional machinery [36]. Histone methyltransferases and demethylases are also critical to conveying information about chromatin accessibility by controlling specific methylation signatures on histone lysine or argenine residues [37,38]. Other histone modifying enzymes which sumoylate [39], phosphorylate [40] and ubiquinate histones [41], although the dynamics of their regulatory ability are less well characterised.

More recently, the roles that non-coding RNAs (ncRNAs) have to play in epigenetic regulation of gene expression have come to light. These are broadly divided into two classes of long and short ncRNAs, and regulate expression both at the pre- and post-transcriptional level. Long ncRNAs are at least 200 nucleotides long [42] and are derived from diverse origins including gene regulatory regions [43], intergenic regions [44] and even mitochondria [45]. Long ncRNAs primarily act locally on the genomic region from which they are derived, attracting transcription factors or epigenetic modifiers. They may also act as precursors to shorter ncRNAs [46]. Conversely, short ncRNAs exert their effects through post-transcriptional regulation of gene expression. Consisting of microRNAs (miRNAs) [47], endogenous short interfering RNAs (endogenous siRNAs) [48] and PIWI interacting RNAs (piRNAs) [49], short-interfering RNAs differ in their origin, processing and structure, but all work post-transcriptionally to repress expression of not only target genes but also other ncRNAs [42].

3. Epigenetic regulation of oestrogen biosynthesis pathways

Before describing the epigenetic alterations mediating oestrogen production, a brief overview of the key players involved in oestrogen biosynthesis is required. The intracrine production of intra-tumoural oestrogen in breast cancer tissue is mediated

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