Molecular and Cellular Endocrinology xxx (2017) 1–5

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

# Mass spectrometry-based metabolic signatures of sex steroids in breast cancer

Man Ho Choi*

*Molecular Recognition Research Center, Korea Institute of Science and Technology, Seoul 02792, South Korea*

ARTICLE INFO

*Article history:*
Received 17 April 2017
Received in revised form
15 September 2017
Accepted 15 September 2017
Available online xxx

*Keywords:*
Sex steroid
Mass spectrometry
Chromatography
Metabolite profiling
Breast cancer

ABSTRACT

Owing to controversy over the effects of steroids on breast cancer pathophysiology, comprehensive quantification of steroid hormones has been extensively considered in both clinical practice and biomarker discovery studies. In contrast to the traditional immunoaffinity-based assays, which show cross-reactivity and have poor validity at low levels of sex steroids, mass spectrometry is becoming a promising tool for measuring steroid levels in complex biological specimens. The Endocrine Society has announced and continuously updated on technical advances to apply high-quality breakthroughs in the clinical sciences. To avoid incorrect estimation of the steroids of interest, however, further emphasis should be made on the efficient separation by chromatography, such as gas and liquid chromatography, prior to mass spectrometric (MS) detection. Recent advances in MS-based analysis of sex steroids associated with breast cancer enable accurate quantification of circulating as well as localized steroids from frozen tissue slices, allowing these assays to be more powerful in clinical practice.



**Contents**



## 1. Introduction

Breast cancer (BCa) risk is associated with reproductive events and lifestyle parameters that lead to altered sex steroid levels. Estrogens play a primary biochemical role in the development and growth of human BCa, which is based on estrogen receptor (ER)-mediated mitogenic and genotoxic effects (Preston-Martin et al., 1990). The levels of endogenous estrogens have a strong relationship with BCa risk (Colditz, 1998) and they are converted into active derivatives catalyzed by hydroxylases and catechol-*O*-methyltransferase, for independent expression in target tissues (Fig. 1).

Hydroxylated estrogens may have an important biochemical role in the onset and clinical progression of BCa. Increased intratumor 16α-hydroxyestrone level was associated with prolonged survival of patients with BCa (Castagnetta et al., 2002), while no association with 2-hydroxyestrone was observed in ER (+)/progesterone receptor (PR) (+) tumors, but a good correlation was observed in ER (−)/PR (−) tumors (Eliassen et al., 2008). In contrast, no significant correlation between BCa risk and serum levels of 2-

* Molecular Recognition Research Center, KIST, 5 Hwarang-ro 14-gil, Seoul 02792, South Korea.
*E-mail address:* mh_choi@kist.re.kr.

http://dx.doi.org/10.1016/j.mce.2017.09.023
0303-7207/

**Abbreviations**

| | |
|---|---|
| BCa | breast cancer |
| ER | estrogen receptor |
| DHT | 5α-dihydrotestosterone |
| DHEA | dehydroepiandrosterone |
| LC-MS | liquid chromatography-mass spectrometry |
| GC-MS | gas chromatography-mass spectrometry |
| CRM | certified reference material |
| ESI | electrospray ionization |
| MS/MS | tandem mass spectrometry |

hydroxyestrone, 16α-hydroxyestrone, and their ratio was reported in a case-control study on premenopausal women (Arslan et al., 2009).

High aromatase activity in BCa results in increased levels of intratumor estrone and 17β-estradiol, which are metabolized from the two androgens, androstenedione and testosterone, respectively (Fig. 1). Therefore, testosterone levels should be monitored together as a part of the prognostic follow-up because increased plasma testosterone may be a good predictor of poorer prognosis in post-menopausal women with BCa (Micheli et al., 2007; Secreto et al., 2009). Serum testosterone levels were also increased in the presence of a metabolic syndrome, which was closely related to BCa progression (Pasanisi et al., 2006). In addition, testosterone rather than 17β-estradiol might be more strongly associated with BCa risk (Cummings et al., 2005).

The metabolic balance between the catechol and methoxylated estrogens as well as between major estrogens and androgens, may affect the BCa risk. Thus, an overview of the sex steroid profile, which provides the metabolic signatures (Ha et al., 2009; Baglietto et al., 2010), is necessary and helps understand the pathophysiological actions of sex steroids in BCa development and treatment. Here, the recent advances in chromatographic separation coupled to mass spectrometric detection of sex steroids over the traditional immunoaffinity-based assays are discussed.

## 2. Analytical issues about steroids

The immunoaffinity-based biochemical assays for analysis of sex steroids, such as testosterone and 17β-estradiol, are simple to perform and have good detection sensitivity (Sikaris et al., 2005; Handelsman et al., 2014). Although they are very useful as a screening method in clinical practice, drawbacks of immunoassays, such as cross reactivity caused by structural similarity and conjugation diversity of steroid backbone, have been well described (Taylor et al., 2015). Testosterone detection using immunoassays is often hampered due to background interference by dehydroepiandrosterone (DHEA) sulfate, which is an abundant steroid in the human serum (Middle, 2007), similar to cortisol precursors obtained from patients administered with metyrapone that interfere in serum cortisol quantification (Monaghan et al., 2011). Immunoaffinity-based assays provide valuable results in primitive experimental conditions without any special requirements, but their variability and specificity should be considered if steroids are present at low levels with matrix interference. As an alternative assay, liquid chromatographic purification prior to radioimmunoassay using estradiol-6-(*O*-carboxymethyl)-oximino-2-(2 [$^{125}$I]-iodo-histamine) as a ligand was firstly introduced to overcome the methodological problems in the low range of estrogens. The method successfully reduced possible cross reactivity by other compounds in breast tissues obtained from patients with malignant (Geisler et al., 2000) and undergoing aromatase inhibitor

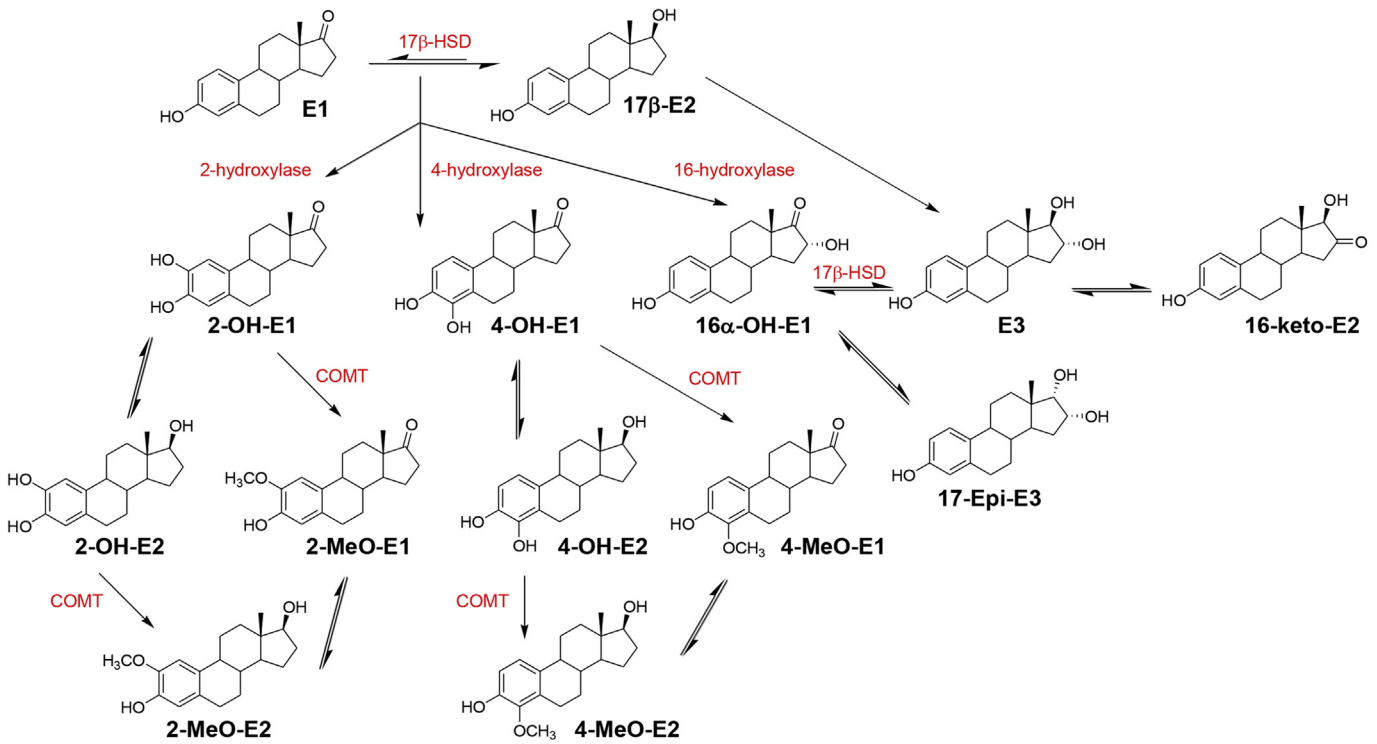


**Fig. 1.** Representative metabolic pathways of estrogens. Major estrogens, estrone and 17β-estradiol, which are synthesized from androstenedione and testosterone, respectively, are hydroxylated at the 2, 4, and 16 carbon positions, to yield 2/4-OH-estrogens and 16α-OH-estrone. Further metabolisms are also catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD) and catechol-O-methyltransferase. E1: estrone; E2: estradiol; E3: estriol.

Please cite this article in press as: Choi, M.H., Mass spectrometry-based metabolic signatures of sex steroids in breast cancer, Molecular and Cellular Endocrinology (2017), http://dx.doi.org/10.1016/j.mce.2017.09.023