The Relevance of Epigenetic Biomarkers for Breast Cancer and Obesity for Personalised Treatment in Public Healthcare: A Systematic Review

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ABSTRACT

Background: Personalised medicine has gained attention as a result of the advances of genomic research in the last decade. This includes the rise in epigenetic research, which focuses on the environmental influences on the genome and examines biomarkers that might be useful for cancer therapy. This study investigates the epigenetic biomarkers for breast cancer and one of its risk factors, obesity, and evaluates their relevance for global public health.
Methods: A systematic search of articles published from 2005 to May 2015 was performed in PubMed for epigenetic breast cancer marker. An additional literature search was carried out on the epigenetic markers of obesity.
Results: The search resulted in 77 articles on breast cancer, which demonstrate the various applications of epigenetic markers for breast cancer diagnostics, prognostics and treatment. Particularly, non-invasive blood-based diagnostic biomarkers and epigenetic therapy could improve the health outcomes of cancer patients using a personalised approach. The 14 obesity-related articles highlight the epigenetic link of disease and risk factors and emphasise the relevance of nutritional influences.
Conclusion: Although epigenetics offers many opportunities, new discoveries have to be confirmed first in clinical settings to ensure advantages over traditional methods. Furthermore, before personalised epigenetic therapy can be applied in public health it is crucial to ensure a fair implementation in both high and low-income settings globally.

Key words: epigenetics, biomarker, breast cancer, personalised medicine, obesity

INTRODUCTION

Technological advances have always influenced progress in public health. In the last decade, the growing research in genomics has led to an anticipated shift in public health towards more personalised therapy approaches. Due to the increase of non-communicable and chronic diseases (NCDs) globally, the cost of healthcare is rising and new prevention and treatment methods gain attention, as vaccines or other traditional drugs fail to help. The economic burden of NCDs is high for both developed and developing countries and challenges the global public health system [1]. New so-called -omic studies, such as genomics, epigenomics, metabolomics or proteomics, are researching the biological conditions and changes related to age, disease or lifestyle and emphasise the individual characteristics of diseases [2]. Biomarkers are a valuable tool to assess biological changes due to age, disease or treatment in a patient and thus play a crucial role for personalised medicine. There are various biomarkers addressing the different levels of carcinogenesis: starting with genetic markers of altered gene expression, protein status and the metabolic level [3]. Compared to others, epigenetic biomarkers can be advantageous as they provide information on the patient's genetic and environmental background [4]. Furthermore, genetic mutation markers are mostly relevant in hereditary diseases whereas epigenetics has a fundamental function in both inherited and sporadic cancers. Epigenomics analyse biological changes by assessing DNA, histone or RNA alterations that can modify gene expression without a shift in the DNA sequence [5]. Research in epigenetic-related diseases and treatment offers great promises for various chronic diseases and offers new personalised therapy options. Although the focus of personalised medicine is on genomic research, the exposome, all non-genetic internal and external influences that are determining a person's health, plays a crucial role in disease development [2]. Epigenetic studies are one way to investigate both the genetic background and the environmental exposures.

This study will focus on the epigenetic biomarker advances in breast cancer and obesity research and will evaluate the opportunities of such biomarkers compared to others. Cancer and obesity are major challenges to global health and cause a high percentage of the global health burden [6,7]. A systematic search of articles will provide an extensive list of suggested biomarkers, which will be assessed for their use and relevance in clinical settings and for global public health. Moreover, epigenetic biomarkers could be useful for diet suggestions and the field of nutriepigenomics is expected to become relevant for improving health and prevent diseases. However, many biomarkers have not yet been sufficiently tested for their accuracy and more research is necessary to ensure that epigenetic biomarkers use will lead to beneficial health outcomes. Furthermore, this study will briefly review personalised medicine and the challenges that have to be overcome before personalised therapy can be applied in clinical public health settings in low and middle income (LMICs) and high-income countries (HICs).

METHODS

A systematic search was carried out collecting literature on epigenetic biomarkers for breast cancer. Additional literature on obesity biomarkers was added to highlight the role of this risk factor. The systematic search included literature from 2005 to May 2015 as this topic is very recent and most relevant literature has been written in the past decade. For breast cancer biomarkers, PubMed was used to search for peer-reviewed literature with at least the abstract available based on the search terms: "epigenetic(s)", "breast cancer" in both title or abstract and "marker(s)" or "biomarker". The search was limited to articles published in English or German and after 2005. The initial PubMed search resulted in 135 articles. Two articles were added to the results of the systematic search after reviewing reference lists of relevant articles and reviews. Due to the focus on prevention and personalised therapy, classification and staging markers investigating either the type of breast cancer or metastases presence were excluded. After applying the exclusion criteria, 81 studies on breast cancer biomarker remained. The literature search on obesity biomarkers was carried out separately. Several articles focused on weight loss success; these were excluded from the study. Only a limited number of obesity-related articles were suitable for comparison with the results of the breast cancer search. Therefore, only 14 articles were included on epigenetic changes associated with a high BMI or weight changes among breast cancer patients.

RESULTS

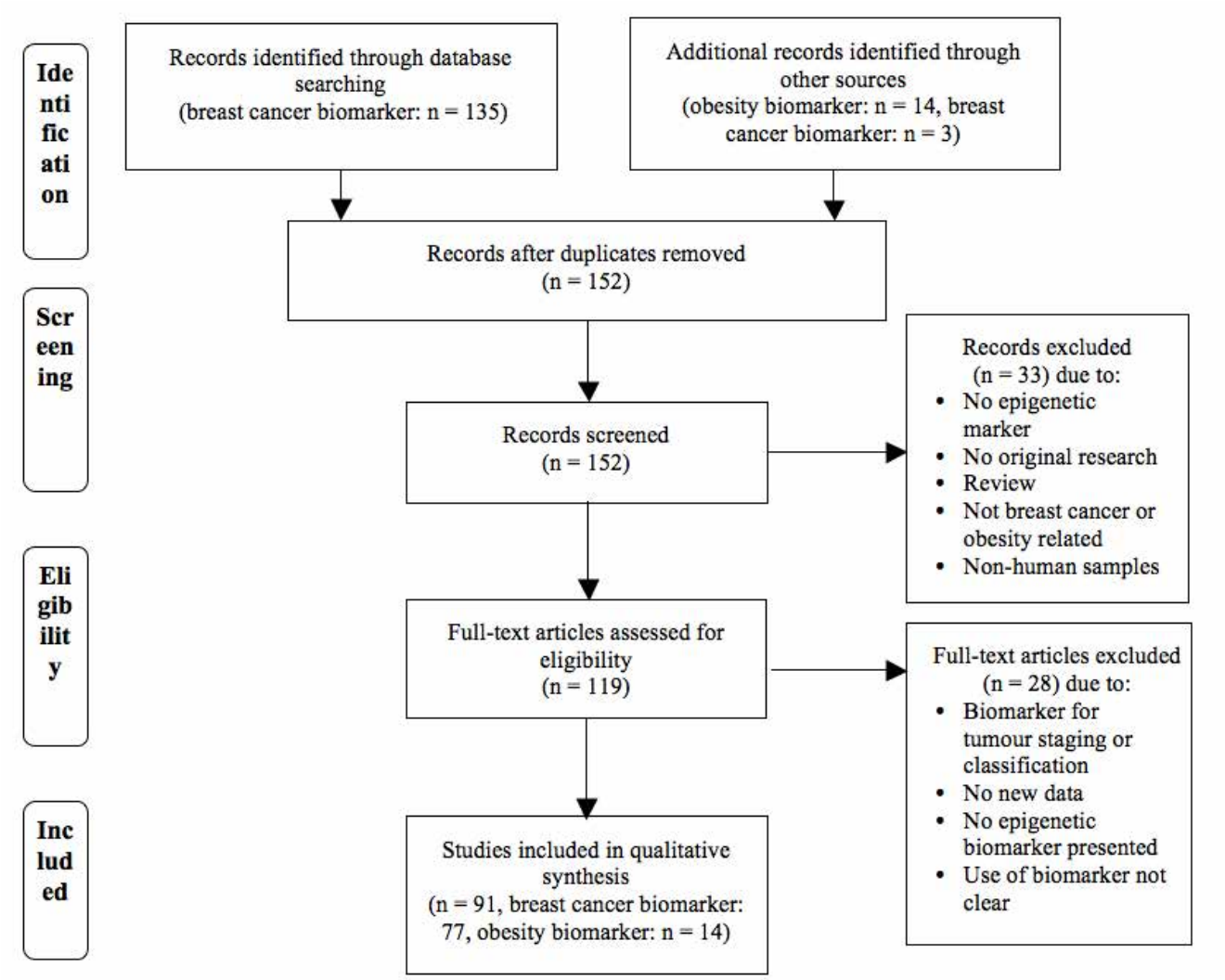
The results of the two literature searches are displayed in a table available as supplementary information. For each publication, the type of biomarker and epigenetic mechanism was noted as well as the main results of the study.

Breast Cancer

The breast cancer biomarkers can be classified into four categories: non-invasive (blood-based) diagnostic, invasive diagnostic, prognostic and therapeutic use.

The diagnostic markers for breast cancer are obtained either invasively by using tumour or breast tissue or non-invasively with the use of body fluids, mainly blood. Twelve studies focused on non-invasive methods of the 34 included diagnostic markers. All

FIGURE 1. PRISMA Flowchart.



studies use either serum or plasma to detect potential biomarkers and emphasise the screening and early diagnostic relevance of the results. Xu et al. find 250 differently methylated CpG sites in breast cancer patients compared to controls, which shows that breast cancer is associated with the DNA methylation profile in blood [8]. For example, Hoque et al. suggest *APC, GSTP1, Rassf1A, and RARbeta2* as diagnostic marker and were able to detect a third of all early stage tumours in their study [9]. Besides blood-based markers, numerous other diagnostic markers have been researched which are found in breast or tumour tissue. Ordway et al. discover 50 methylation biomarkers in tumour breast tissue, which could be relevant for diagnostic use [10]. The majority of the articles focus on single marker for diagnostic use such as *BRCA1, GHSR, or MGMT methylation* [11-13].

The prognostic biomarkers discovered in the literature are either associated with tumour aggressiveness, risk for relapse or poor survival. The hypermethylation of ER-alpha is the only blood-based prognostic marker [14]. Lower survival rates and relapse risk are associated for example with GASC1-negative types by histone demethylase [15].

The epigenetic treatment biomarker studies aim to reverse epigenetic mechanisms and thus improve sensitivity to other therapy options or reduce cancer progression. Radpour et al. show that demethylation therapy could change the expression of several tumour suppressors (including *CDKN1A, PTEN, CST6, BRCA1 and RASSF1*) and oncogenes (such as *ETSA, CXCL1, CXCL2, CXCL3, ERBB2*), as well as affect miRNA and protein expression [16]. Several studies focus on the epigenetic reversal of certain genes to improve survival, re-express tumour suppressor genes or loss of cell growth. Other research teams focus on the epigenetic reversal of gene expression to increase sensitivity to hormonal treatment with tamoxifen and trastuzumab. In other experiments, oestrogen-receptor-negative breast cancer (ER) is modified with the

use genistein, arsenic trioxide and valproic acid to improve sensitivity to endocrine therapy [17–19]. Two research teams focus on biomarkers for treatment response instead of epigenetic therapy [20,21]. Ai et al. show that *TGM2* expression is associated with chemotherapy response and can be demethylated to increase sensitivity [21].

Obesity

The literature on obesity marker aimed to discover epigenetic markers, which are associated with weight gain or loss and breast cancer. For example, *IL-6* methylation is correlated with body weight [22]. Interestingly, hypermethylation of important tumour suppressors such as *RASSF1A* and *BRCA1*, which are frequently silenced in breast cancer, is associated with being overweight [23]. Physical activity was related with ER and PR-positive cancer and methylation of *GSTP1*, which has been suggested as a diagnostic marker due to its role in cancers [9,24]. Furthermore, McCullough et al. demonstrate that global DNA methylation is associated with postmenopausal breast cancer risk and BMI or physical activity [25].

DISCUSSION

The literature search demonstrates the various opportunities of epigenetic biomarker or breast cancer treatment. Particularly the non-invasive diagnostic marker and the epigenetic therapy options offer potential for novel personalised screening and treatment methods. The obesity markers highlight the epigenetic link of risk factor and disease and indicate the impact of nutritional factors on a patient's health. Although these studies present numerous possibilities, none of the suggested biomarkers can be used in breast cancer treatment yet.

Similar to the results of this literature search, DNA methylations found in serum have been highlighted as a way to screen for and detect breast cancer in other studies [26,27]. However, some of these diagnostic biomarkers, such as *RASSF1A* or the expression of other tumour suppressors, are related to various cancers and their epigenetic modification does not necessarily indicate a breast tumour. Thus, it has been suggested that methylation can detect cancer but it is not specific enough to test for specific cancer types [28]. Furthermore, it is important to consider that normal cells may show altered methylation without a disease present. This led van der Vaart and Pretorius to conclude that DNA methylation in blood is currently overrated as a cancer biomarker [28]. Yet, current breast cancer screenings benefit only 3-13% of patients and tumours are not picked up if they develop during screening intervals. Furthermore, over-diagnosis of harmless or slow growing tumours can be estimated to be around 35% [27]. Therefore, diagnostic biomarkers could help to develop better screening methods for early and accurate tumour detection.

A way to improve screening is to establish a set of markers by combining genomic information. An optimal set of biomarkers would include not just epigenetic information but combine genetic, noncoding RNA and other markers. By combining cross-disciplinary research, it would be possible to detect breast cancer with greater certainty. According to Van De Voorde et al. [26], a panel of biomarkers is essential to achieve a higher sensitivity with high specificity. A study, which included almost 55,000 papers from PubMed on 36 types of cancer, aimed to find such a set of markers to distinguish between cancer types [29]. The result was a combination of ten markers, which were able to differentiate 35 out of 36 types of cancer; only penile and testicular cancer could not be distinguished. Future research should thus focus on developing a standardised set of markers, which can detect breast cancer through early blood-based screenings.

Despite the numerous possibilities epigenetic therapy holds, its implementation in public health settings comes with challenges. Demethylating agents may lack specificity and could affect multiple genes besides the targeted onco- or tumour suppressor genes [30]. Furthermore, DNA methylation is a normal process and the reversal of gene expression may have unexpected results [5,30]. Methylation can occur randomly without indicating tumour development; this could lead to overdiagnosis [30]. It is thus necessary to analyse each marker case by case and assess its relevance for public health.

The literature search on obesity marker demonstrates that epigenetic alterations caused by a high BMI may affect tumour-related genes and thus increase breast cancer risk [22–24]. Due to the impact of nutrition on health through epigenetic mechanisms, the field nutriepigenomics has gained attention. This field is linked to nutrigenomics, which focuses on how an individual's genetic make-up is influenced by diet and how health can be improved by making dietary adjustments [31,32]. High-fat diets have been shown to be positively associated with risk of developing cancer for over two decades [33]. Although additional factors such as lifestyle and BMI could interact with cancer risk, obesity, which is often connected with a high fat and Western diet, has widely been accepted as a major risk factor for breast cancer [33,34]. Another nutritional factor is genistein from soybeans, which has been shown to epigenetically affect silenced tumour suppressors and has been demonstrated to reactivate *p16INK4, RARbeta and MGMT* [31]. Li et al. suggest the use of genistein in combination with anti-oestrogen therapy for breast cancer patients [18]. Although high soy consumption seems to have a preventive effect in Asian populations, its impact for breast cancer therapy requires further research [18,33]. Nutriepigenomics should not be understood as a treatment method on its own but rather as a support to other treatment approaches or to be applied for preventive use. Lindroth estimates that 80% of chronic diseases can be prevented through healthy diet and lifestyles [31]. As

chronic diseases are becoming increasingly burdensome worldwide, personalised nutrition could be a crucial factor to improve health and prevent NCDs [31,32]. Clinical settings should thus focus on a cross-disciplinary approach and include nutritional factors, which may help the patient along with established therapy methods.

In the literature, the opportunities of epigenetics for breast cancer are mostly demonstrated in combination with the possibilities of personalised medicine. Napieralski even states that "[they] believe that within a few years no breast cancer patient will receive anything other than tailored cancer therapy to allow personalised cancer care" [35]. Personalised approaches offer opportunities as standardised treatment options can be a problem with some drugs working for only 30% of all patients and with some medication causing adverse drug reactions [36]. Thus, genomic medicine has been widely debated as a revolutionising shift for public health, which will help to cope with the increasing burden of NCDs [37]. Yet, there has also been criticism as most funding for genomic health goes into the initial stage of discovery but not into the application of this knowledge [38]. This can also be seen in the results of this literature analysis. None of the studies presents an established diagnostic biomarker that can accurately detect breast cancer. It is thus important to move away from novel discoveries and towards the next stages of genomic research and to establish evidence for the effectiveness of the proposed markers. Another challenge of personalised medicine is to make it a global public health goal and share the research information with poorer countries [38]. Supporters of individualised treatment even argue that this approach could become more economical than traditional medicine and could thus significantly help LMICs [39,40]. Therefore, several steps have to be taken before epigenetic markers can benefit global public health. Firstly, a set of markers for diagnosis has to be established so that the results of such markers can accurately indicate breast cancer. Secondly, a multidisciplinary approach is necessary to validate such a set and make use of genetic, epigenetic and other genomic knowledge. This cross-disciplinarity is also important to assess potential risk factors. Thirdly, the impact of nutrition is only one aspect that can influence breast cancer risk, other environmental factors such as lifestyle or environmental toxins should be considered. Brand emphasises the importance of "the shift in healthcare towards a systemic and holistic understanding of the aetiology of diseases or health outcomes ('systems thinking')" as a scientific revolution [41]. This approach will make it possible to improve disease prevention and combine traditional medicine with genomic information.

A limitation of this study is that it only provides an overview of the research done in the last ten years. Searching for peer-reviewed articles on biomarkers may also overstate their relevance as studies with positive results are more often published. It is possible that research, which could not present positive results regarding the use of biomarkers, is missing from these results. Due to the amount of results from the literature search, articles cannot be reviewed individually for their quality and relevance. Therefore, it will be necessary for future studies to highlight the advantages of specific markers compared to others. This will include the focus on clinical trials of such biomarkers and their effect on a patient. Only by bringing research further towards clinical trials, it will be possible to investigate the potential use of epigenetic screening or treatment approaches.

The literature search demonstrated the varied use of epigenetic markers for breast cancer treatment. The studies included numerous ways to help patients by potentially improving diagnostics, prognostics and treatment. Furthermore, looking at the obesity biomarkers demonstrates that this risk factor is clearly linked to breast cancer through epigenetic mechanisms. This highlights the impact of lifestyle factors such as diet on cancer risk and emphasises the applicability of nutriepigenomics. The personal genomic background could thus inform patients on dietary choices to improve their health. This personal approach will also play a crucial role in therapy and in the development of personalised medicine approaches. The shift towards personalised healthcare could bring a range of opportunities for public health. Yet, before the implementation of individualised methods, it is important to test the use of epigenetic biomarker in clinical trials. For global health, it is important that such medical advances reach both HICs and LMICs to improve health equally.

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Ethical approval was not required as this study is based on already published data.

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References

1. Bloom DE, Cafiero E, Jané-Llopis E, et al. The Global Economic Burden of Noncommunicable Diseases. Program on the Global Demography of Aging, 2012.
2. Wild CP. The exposome: from concept to utility. Int J Epidemiol 2012;41:24–32.
3. Bhatt AN, Mathur R, Farooque A, Verma A, Dwarakanath BS. Cancer biomarkers-Current perspectives. Indian J Med Res 2010;132:129.
4. Verma M, Khoury MJ, Ioannidis JPA. Opportunities and Challenges for Selected Emerging Technologies in Cancer Epidemiology: Mitochondrial, Epigenomic, Metabolomic, and Telomerase Profiling. Cancer Epidemiol Biomarkers Prev 2013;22:189–200.
5. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004;429:457–63.

6. Crujeiras AB, Casanueva FF. Obesity and the reproductive system disorders: epigenetics as a potential bridge. Hum Reprod Update 2014;21:249–61.
7. Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. Endocr Relat Cancer 2010;17:R245–62.
8. Xu Z, Bolick SCE, DeRoo LA, et al. Epigenome-wide association study of breast cancer using prospectively collected sister study samples. J Natl Cancer Inst 2013;105:694–700.
9. Hoque MO, Feng Q, Toure P, et al. Detection of aberrant methylation of four genes in plasma DNA for the detection of breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 2006;24:4262–9.
10. Ordway JM, Budiman MA, Korshunova Y, et al. Identification of novel high-frequency DNA methylation changes in breast cancer. PloS One 2007;2:e1314.
11. Botla SK, Gholami AM, Malekpour M, et al. Diagnostic values of GHSR DNA methylation pattern in breast cancer. Breast Cancer Res Treat 2012;135:705–13.
12. Fumagalli C, Pruneri G, Possanzini P, et al. Methylation of O6-methylguanine-DNA methyltransferase (MGMT) promoter gene in triple-negative breast cancer patients. Breast Cancer Res Treat 2012;134:131–7.
13. Stefansson OA, Jonasson JG, Olafsdottir K, et al. CpG island hypermethylation of BRCA1 and loss of pRb as co-occurring events in basal/triple-negative breast cancer. Epigenetics Off J DNA Methylation Soc 2011;6:638–49.
14. Hagrass HA, Pasha HF, Ali AM. Estrogen receptor alpha (ER) promoter methylation status in tumor and serum DNA in Egyptian breast cancer patients. Gene 2014;552:81–6.
15. Berdel B, Nieminen K, Soini Y, et al. Histone demethylase GASC1- -a potential prognostic and predictive marker in invasive breast cancer. BMC Cancer 2012;12:516.
16. Radpour R, Barekati Z, Kohler C, et al. Integrated epigenetics of human breast cancer: synoptic investigation of targeted genes, microRNAs and proteins upon demethylation treatment. PloS One 2011;6:e27355.
17. Du J, Zhou N, Liu H, et al. Arsenic induces functional re-expression of estrogen receptor by demethylation of DNA in estrogen receptor-negative human breast cancer. PloS One 2012;7:e35957.
18. Li Y, Meeran SM, Patel SN, et al. Epigenetic reactivation of estrogen receptor- (ER) by genistein enhances hormonal therapy sensitivity in ER-negative breast cancer. Mol Cancer 2013;12:9.
19. Travaglini L, Vian L, Billi M, Grignani F, Nervi C. Epigenetic reprogramming of breast cancer cells by valproic acid occurs regardless of estrogen receptor status. Int J Biochem Cell Biol 2009;41:225–34.
20. Halvorsen AR, Helland A, Fleischer T, et al. Differential DNA methylation analysis of breast cancer reveals the impact of immune signaling in radiation therapy. Int J Cancer J Int Cancer 2014;135:2085–95.
21. Ai L, Kim W-J, Demircan B, et al. The transglutaminase 2 gene (TGM2), a potential molecular marker for chemotherapeutic drug sensitivity, is epigenetically silenced in breast cancer. Carcinogenesis 2008;29:510–8.
22. Aumueller E, Remely M, Baeck H, et al. Interleukin-6 CpG Methylation and Body Weight Correlate Differently in Type 2 Diabetes Patients Compared to Obese and Lean Controls. J Nutr Nutr 2015;8:26–35.
23. Naushad SM, Hussain T, Al-Attas OS, et al. Molecular insights into the association of obesity with breast cancer risk: relevance to xenobiotic metabolism and CpG island methylation of tumor suppressor genes. Mol Cell Biochem 2014;392:273–80.
24. McCullough LE, Chen J, White AJ, et al. Gene-Specific Promoter Methylation Status in Hormone-Receptor-Positive Breast Cancer Associates with Postmenopausal Body Size and Recreational Physical Activity. Int J Cancer Clin Res 2015;2.
25. McCullough LE, Chen J, White AJ, et al. Global DNA Methylation, Measured by the Luminometric Methylation Assay (LUMA), Associates with Postmenopausal Breast Cancer in Non-Obese and Physically Active Women. J Cancer 2015;6:548–54.
26. Van De Voorde L, Speeckaert R, Van Gestel D, et al. DNA methylation-based biomarkers in serum of patients with breast cancer. Mutat Res 2012;751:304–25.
27. Wittenberger T, Sleigh S, Reisel D, et al. DNA methylation markers for early detection of women's cancer: promise and challenges. Epigenomics 2014;6:311–27.
28. van der Vaart M, Pretorius PJ. Is the role of circulating DNA as a biomarker of cancer being prematurely overrated? Clin Biochem 2010;43:26–36.
29. Razvi E, Oosta G. Eye on Cancer Biomarkers. Genet Eng Biotechnol News 2013.
30. Weber WW. The promise of epigenetics in personalized medicine. Mol Interv 2010;10:363–70.
31. Lindroth AM, Park JH, Yoo Y, Park YJ. Nutriepigenomics : Personalized nutrition meets epigenetics. In: Tollefsbol T, editor. Pers. Epigenetics, San Diego: Academic Press; 2015.
32. Remely M, Stefanska B, Lovrecic L, Magnet U, Haslberger AG. Nutriepigenomics: the role of nutrition in epigenetic control of human diseases. Curr Opin Clin Nutr Metab Care 2015;18:328–33.
33. Teegarden D, Romieu I, Lelièvre SA. Redefining the impact of nutrition on breast cancer incidence: is epigenetics involved? Nutr Res Rev 2012;25:68–95.
34. World Cancer Research Fund, American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective, 2007.
35. Napieralski R, Brünner N, Mengele K, Schmitt M. Emerging biomarkers in breast cancer care. Biomark Med 2010;4:505–22.
36. Kaur JS, Petereit DG. Personalized Medicine: Challenge and Promise. J Cancer Educ 2012;27:12–7.
37. Brand A. Health care faces a paradigm shift. Bayer Sci Mag, 2011.
38. Burke W, Burton H, Hall AE, et al. Extending the reach of public health genomics: What should be the agenda for public health in an era of genome-based and "personalized" medicine? Genet Med 2010;12:785–91.
39. Abrahams E, Silver M. The case for personalized medicine. J Diabetes Sci Technol 2009;3:680–4.
40. Jain KK. Textbook of Personalized Medicine. New York, NY: Springer New York, 2009.
41. Brand A. Public Health Genomics— public health goes personalized? Eur J Public Health 2011;21:2–3.