Peer Review File

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Comment 1: In the abstract, please indicate why there is a need for this review topic and its potential clinical significance. The main findings part of abstract should have a summary of the most recent and significant findings from available literature. In the conclusion part of the abstract, I suggest the authors to have comments on clinical implications and problems to be addressed in the future.

Reply 1: We appreciate the comments by reviewer and have made the following revisions accordingly. The abstract has been improved based on the valuable advice of the reviewer (Page 2, Line 6-19).

Changes in the text: 'Conclusions: Cancer initiation and progression are controlled by both genetic and epigenetic events. Unlike genetic changes, epigenetic modulations are potentially reversible. Epigenetic drugs that inhibit DNA methylation or histone deacetylation enable reactivation of tumor suppressor genes and suppression of cancer cell growth. Taking advantage of these situations allows the reduction of malignant cell clusters. In addition, clinical results, such as epigenetic drugs targeting specific enzymes for cancer treatment and re-sensitizing cells that do not respond to treatment are promising as cancer therapeutics. To date, numerous epigenetic agents have been developed, several DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors have been definitively approved by regulatory agencies. Combined multidrug approaches for cancer treatment have overcome the limitations of single-agent epigenetic therapies, increased antitumor effects, and reduced drug resistance. It is evident that as our knowledge on epigenetic mechanisms expand, epigenomics-targeted treatments will become more common in cancer therapy, either as primary therapy or as complementary and alternative treatment options to increase the efficacy of conventional treatments for cancer patients. All these developments show that epigenetics will maintain its increasing importance for cancer diagnostic, prognostic and therapeutic studies for many years to come.'

Comment 2: The introduction part should focus on why there is a need for a review of epigenetic mechanisms in cancer and the possible clinical significance.

Reply 2: We appreciate the comments by reviewer and we have made the following revisions accordingly. The text is improved based on the valuable advice of the reviewer (Page 4, Line 7-18).

Changes in the text: 'The increase of knowledge on epigenetics resulted in its reflection on the clinic with new diagnostic and therapeutic strategies. Epigenetics not only offers new insights into the changes in gene regulation that occur during the disease process, but also provides the basis for epigenomics-based targeted therapies. There are many agent currently being tested in clinical trials and some of them are already in clinical use. In addition, drug research and development studies carried out to correct epigenetic errors have gained great speed in recent years. Targeting HDACs and DNA/histone methyltransferases (DNMT/HMT) are used as possible targets in the treatment of various types of cancer. There are FDA-approved histone deacetylase inhibitors and DNA methylation inhibitors. Their clinical use gives successful results. Apart from these, histone methylation and microRNAs have also attracted attention as potential therapeutic targets. Combined treatment options of standard chemotherapeutic drugs with epigenetic targeting drugs make it possible to reactivate genes sensitive to chemotherapeutic drugs. It is thought that epigenetic studies will continue for many years and will provide indispensable advantages in many diseases.'

Comment 3: In the part of literature review, please also have a comment on the methodology of studies reviewed.

Reply 3: In our study, an integrative approach was followed, since the perspectives and data of different fields and research groups on epigenetic changes were combined. A short paragraph has been added to the end of the literature section. We have made the following revisions accordingly. (Page 4, Line 22-25).

Changes in the text: 'This review aims to give a brief summary of the most common epigenetic mechanisms, their possible relations with cancer initiation and progression, focusing on the possible physico-chemical factors that might control these epigenetic mechanisms, and giving examples of the epigenetic therapy approaches. Research articles, books, and other published texts were examined using integrative methodology.'

Comment 4: In the discussion, the authors may consider to have comments on problems to be addressed and suggest how to address this issues in the future. You may consider several comments on the clinical treatment from the translational perspective.

Reply 4: We appreciate the comments by reviewer and have made the following revisions accordingly. The Conclusions and Future Perspectives section has been improved based on the valuable advices of the reviewer (Page 22, Line 11 – Page 23, Line 6).

Changes in the text: 'In addition to all its advantages, it can be difficult to work with epigenetic modifications. When DNA methylation biomarkers are analyzed, cell heterogeneity found in tissues from clinical specimens may yield variable, dynamic and complex profiling results. These variable results have to be interpreted in the context of real time changes in the tissues which may in turn influence the epigenetic modifications, which are highly susceptible to local factors, such as cell metabolism, oxygenation, free radical formation and inflammation. In addition, normal cell density is low in body fluids like serum, plasma, urine and sputum, which may make it difficult to detect epigenetic biomarkers in rare cancer cells. However, new highly sensitive cell identification and imaging technologies allow for accurate analysis of single cancer cells found in tissues and circulation, as well as different cell populations in the microenvironment. New methodologies also allow for "liquid biopsies" by identifying cell free DNA in the circulation. It is now possible to get multiple blood and tissues samples during the course of cancer treatment to evaluate genetic and epigenetic changes, which may influence the selection of different anti-cancer treatments.

Heterogeneity in differentiation status in cancerous cells and changes in the histological grade of the tumor over the course of the disease may cause ambiguous results in the correlation of clinical status. While the treatment processes of the disease are being followed, uncertain results may be obtained in patient samples Therefore, clinicians may have to obtain serial blood and tissues samples to understand the changes in genetic and epigenetic profile of the disease, and make necessary treatment changes accordingly. For example, due to the working principle of miRNAs, a miRNA has hundreds of targets. This may cause irregular treatment responses and different pathological processes that appear as a complex network.

In order to overcome the possible problems mentioned above, it is necessary to work in tissues and body fluids with high stability, to purify them at the DNA methylation stage, to create large patient groups, and to use biomarker panels approved by different regulatory agencies, such as FDA or EMA. Also, a good epigenetic biomarker should be more cost-effective than some of the existing markers.'