DR ALFRED GRECH & DR MICHAEL BALZAN

Cancer Epigenetics and its Clinical Applications

ABSTRACT

Epigenetics is defined as 'somatically inheritable changes that are not accompanied by alterations in DNA sequence'. DNA methylation, histone modifications and non-coding RNAs are all epigenetic mechanisms. Epigenetic aberrations (or epimutations) have been found to be responsible for carcinogenesis. The research of these epimutations is providing a platform for their application in cancer diagnosis, prognosis and therapeutics.

INTRODUCTION

What causes cancer? One of the most frequent answers to this question is that 'it's in our genes', but what does that mean? The wealth of literature demonstrating that damaged genes are a cause of cancer is irrefutable. However, in recent years, a growing body of research has suggested that epigenetics also plays a critical role in this disease.[1]

Historically, the word 'epigenetic' was used to describe the phenomenon of cell identity.[2] Our bodies exhibit a great diversity in cell identity, with over 200 different cell types all depending on one genome. This variation is regulated by a system of biochemical alterations of DNA and histone proteins, which give DNA its structure. Together, these modifications are termed the epigenome.

Epigenetic modifications include DNA methylation, histone modifications and RNA epigenetics. Epigenetic modifications have importance in gene transcription, but they do not actually modify the coding sequence of the gene itself. While these alterations are heritable, there is also the possibility of reversing them; a fact that has enabled the prospect of epigenetic therapy in cancer treatment. Indeed, epimutations have therefore been targeted for new drug innovations, and "turning back on" silenced genes represents a crucial advancement in treating different forms of cancer.[3]

EPIMUTATIONS IN CANCER

DNA methylation

DNA methylation is a type of epigenetic modification that has been extensively studied in mammals. In normal cells, it ensures the correct regulation of gene expression and stable gene silencing. DNA methylation is closely linked with histone modifications, and their interaction is fundamental in controlling genome functioning by altering chromatin architecture. Generally, the methylation pattern is maintained by DNA methyltransferases. In various cancer types, DNA methylation has been shown to silence a wide range of genes, with inactivation of certain tumour-suppressor genes occurring as a consequence of hypermethylation within the promoter regions.[4]

Histone modifications

In normal cells, histone modifications systematically coordinate and oversee cellular processes such as DNA repair, DNA replication and gene transcription. In recent years, research attention on histone modifications has intensified, leading to the detection and categorisation of many histone-modifying protein complexes and molecules. Changes in these complexes are thought to disturb the configuration and levels of histone marks, and so disrupt the regulation of chromatin-based processes, eventually resulting in oncogenic transformation and cancer development.[5]

RNA epigenetics

Several studies have shown that microRNAs (miRNAs) can also have clinical relevance as biomarkers to indicate the presence of a pathology and also the genetic link, progression or stage of the cancer.[6]

It is known that epigenetic factors are involved in aberrations of the miRNome (that is, the complete set of miRNAs for a certain genome) which are seen in cancer. It is also known that a group of miRNAs known as epi-miRNAs can directly target effectors of the epigenetic machinery, including DNA methyltransferases, histone deacetylases (HDACs), and polycomb repressive complex genes. Epi-miRNAs can also indirectly affect the expression of tumour suppressor genes, whose expression is controlled by epigenetic factors.

Such epigenetic-miRNA interaction results in a new layer of complexity in gene regulation, opening up new avenues in the understanding of human cancerogenesis and in the achievement of new cancer treatments.[7]

EPIGENETIC THERAPY OF CANCER

In a race against precious time, epigenetic therapy emerged as a treatment option when it was discovered that the epigenetic alterations that occur in cancer are reversible in nature. In fact, one of the main aims of epigenetic therapy is to reverse the epigenetic modifications that occur in cancer, therefore leading to the restoration of a healthy and normal epigenome. In recent years, drug development on this front has focused on DNA methyltransferases, HDACs, histone acetyltransferase (HATs) and miRNA-based therapeutic strategies.[8]

DNA methyltransferase inhibitors

Drugs such as the DNA methyltransferase inhibitors azacitidine[9] and decitabine[10] have, for instance, been found to target the inverted methylation pattern of cancer cells. It was shown that these hypomethylating agents not only inhibit all three types of DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b), but they are also effective when used in low dosage. Indeed, both azacitidine and decitabine have been used in the treatment of patients with acute myeloid leukaemia, chronic myelomonocytic leukaemia and higher-risk myelodysplastic syndromes, who are unable to be considered for more intensive treatments such as induction chemotherapy or stem cell transplantation.[11]

HDAC inhibitors

In addition to DNA methyltransferase inhibitors, a few HDAC inhibitors have been approved by the FDA, including vorinostat, panobinostat and romidepsin. Besides the histone of interest, these HDAC inhibitors are known to alter the acetylation state of many proteins. Additional research at the molecular level of patient response is therefore needed to improve the effectiveness of these inhibitors as cancer treatment. Nonetheless, both vorinostat and romidepsin have been used in the treatment of cutaneous T-cell lymphoma.[12,13] Similarly, panobinostat has been used as a therapeutic option for multiple myeloma, although its use has been restricted due to its adverse event profile.[14]

HAT inhibitors

HAT inhibitors are other pharmaceutical targets in cancer research. Lunasin, for example, is a soybean-derived polypeptide which has been found to bind to deacetylated histones competing with HATs, and in doing so, switching off transcription. Such an epigenetic mechanism therefore suggests that this polypeptide can affect regulatory pathways involving chromatin changes that could be important to carcinogenic pathways. Wan et al. (2017) argue that lunasin could therefore be effective against cancers that involve chromatin modifications.[15]

Similarly, the natural product HAT inhibitors anacardic acid[16] and garcinol[17] have been shown to sensitise cancerous cells to irradiation. In mice, garcinol inhibited proliferation of breast cancer cells and also suppressed colon carcinogenesis.[18,19] Curcumin, which is another HAT inhibitor, is currently in clinical trials as a therapeutic agent and combination therapy, however its biological effect is not only due to its HAT inhibition.[20]

miRNA-based therapeutic strategies

Epigenetic therapies also include miRNA-based therapeutic strategies. In general, miRNAs are a feasible therapeutic tool because they can regulate key cellular processes by also simultaneously regulating several targets. Currently, there are two strategies for the treatment of cancer using RNAi-based therapy: (a) the 'sandwich RNAi inhibition' strategy and (b) the 'multiplex RNAi inhibition' strategy.[21,22] In the former strategy, multiple agents are used to target a specific molecular defect connected to cancer pathogenesis. In the latter strategy, on the other hand, it is the various molecular defects which accumulate in the multistep pathway of a specific cancer that are targeted.

In murine models for example, Xue et al. (2014) found that delivery of miR-34a and K-ras siRNA into a lung cancer model resulted in significant tumour regression.[23] Similarly, Yuan et al. (2014) found that siRNA-mediated inhibition of KRAS, in addition to RAF or PI3K combinations, could impair KRAS-mutant colorectal cancer in xenograft models.[24]

In human studies, while some miRNA candidates have failed, others have shown potential. In the first human trial of its kind, a technology termed "TargomiR" exhibited promising results in patients with malignant pleural mesothelioma and non-small cell lung cancer.[25] In this technology, miRNA mimics are delivered by targeted bacterial minicells. In addition, there is a new miRNA drug candidate called RGLS5579 that targets miR-10b which has potential in patients diagnosed with glioblastoma multiforme – one of the most aggressive forms of brain cancer.[26,27] In glioblastomas, miR-10b is over-expressed and is an oncogenic miRNA. Overall, these studies suggest a feasible future for miRNA drugs in cancers with no effective treatments.

CONCLUSION

It is clear that epigenetic therapeutics has a promising future for cancer treatment. Nevertheless, we still need to fully comprehend the complex system of interactions between the human genome and epigenome, transcriptome and proteome. Looking into the future, if the potential for epigenetic therapy is fulfilled, it will definitely open an exciting new avenue for personalised cancer treatment medicines.

REFERENCES

1. Issa JJ. Introduction: Cancer as an Epigenetic Disease. *Cancer J* 2017;23(5):255-256.
2. Slack JM. Conrad Hal Waddington: the last Renaissance biologist? *Nat Rev Genet* 2002;3(11):889-95.
3. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010;31(1):27-36.
4. Kulis M, Esteller M. DNA methylation and cancer. *Adv Genet* 2010;70:27-56.
5. Sawan C, Herceg Z. Histone modifications and cancer. *Adv Genet* 2010;70:57-85.
6. Hanna J, Hossain GS, Kocerha J. The Potential for microRNA Therapeutics and Clinical Research. *Front Genet* 2019; 10:478.
7. Fabbri M, Calin GA. Epigenetics and miRNAs in human cancer. *Advances in Genetics* 2010;70:87-99.
8. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov* 2006;5(1):37-50.
9. Shapiro RM, Lazo-Langner A. Systematic review of azacitidine regimens in myelodysplastic syndrome and acute myeloid leukemia. *BMC Hematol* 2018;18:3.
10. He PF, Zhou JD, Yao DM, et al. Efficacy and safety of decitabine in treatment of elderly patients with acute myeloid leukemia: A systematic review and meta-analysis. *Oncotarget* 2017;8(25):41498-41507.
11. Derissen EJ, Beijnen JH, Schellens JH. Concise drug review: azacitidine and decitabine. *Oncologist* 2013;18(5):619-24.
12. Olsen EA, Kim YH, Kuzel TM, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 2007;25(21):3109-15.
13. Reddy SA. Romidepsin for the treatment of relapsed/refractory cutaneous T-cell lymphoma (mycosis fungoides/Sézary syndrome): Use in a community setting. *Crit Rev Oncol Hematol* 2016;106:99-107.
14. Laubach JP, Moreau P, San-Miguel JF, et al. Panobinostat for the Treatment of Multiple Myeloma. *Clin Cancer Res* 2015;21(21):4767-73.
15. Wan X, Liu H, Sun Y, et al. Lunasin: A promising polypeptide for the prevention and treatment of cancer. *Oncol Lett* 2017;13(6):3997-4001.
16. Sun Y, Jiang X, Chen S, et al. Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation. *FEBS Lett* 2006;580(18):4353-6.
17. Oike T, Ogiwara H, Torikai K, et al. Garcinol, a histone acetyltransferase inhibitor, radiosensitizes cancer cells by inhibiting non-homologous end joining. *Int J Radiat Oncol Biol Phys* 2012;84(3):815-21.
18. Ye X, Yuan L, Zhang L, et al. Garcinol, an acetyltransferase inhibitor, suppresses proliferation of breast cancer cell line MCF-7 promoted by 17β-estradiol. *APJCP 2014;15(12):* 5001-7.
19. Tsai ML, Chiou YS, Chiou LY, et al. Garcinol suppresses inflammation-associated colon carcinogenesis in mice. *Mol Nutr Food Res* 2014;58(9):1820-9.
20. Priyadarsini KI. The chemistry of curcumin: from extraction to therapeutic agent. *Molecules* 2014;19(12):20091-112.
21. Shah MY, Ferrajoli A, Sood AK, et al. microRNA Therapeutics in Cancer - An Emerging Concept. *EBioMedicine* 2016;12:34-42.
22. Calin GA, Croce CM. Chronic lymphocytic leukemia: interplay between noncoding RNAs and protein-coding genes. *Blood* 2009;114(23):4761-70.
23. Xue W, Dahlman JE, Tammela T, et al. Small RNA combination therapy for lung cancer. *Proc Natl Acad Sci USA* 2014;111(34):E3553-61.
24. Yuan TL, Fellmann C, Lee CS, et al. Development of siRNA payloads to target KRAS-mutant cancer. *Cancer Discov* 2014;4(10):1182-1197.
25. Reid G, Kao SC, Pavlakis N, et al. Clinical development of TargomiRs, a miRNA mimic-based treatment for patients with recurrent thoracic cancer. *Epigenomics* 2016;8(8):1079-85.
26. Ghosh D, Nandi S, Bhattacharjee S. Combination therapy to checkmate Glioblastoma: clinical challenges and advances. *Clin Transl Med* 2018;7(1):33.
27. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). May 2013. Identifier NCT01849952, Evaluating the Expression Levels of MicroRNA-10b in Patients With Gliomas. Cited 17 Sep 2020. Available from: www.clinicaltrials.gov/ct2/show/NCT01849952.