Epigenetic Biomarkers in Cancer

Ramón Cacabelos[1,2]*

[1]*Genomic Medicine, Camilo José Cela University, Madrid, Spain*
[2]*EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, Corunna, Spain*

The Holy Grail of medical diagnosis is the identification of specific biomarkers for a predictive diagnosis or the accurate diagnostic confirmation of a pathologic process. Over the past decade, abundant data have confirmed that important epigenetic changes are at the base of many oncogenic processes and that different epigenetic signature might be of clinical utility as biomarkers of cancer.

Epigenetics, defined as phenotypic changes transmitted from one generation to another with no apparent alterations in structural DNA, is a common phenomenon in health and disease. Classical epigenetic mechanisms, including DNA methylation, histone modifications, and microRNA (miRNA) regulation, are among the major regulatory elements that control metabolic pathways at the molecular level. These epigenetic modifications regulate gene expression transcriptionally, and miRNAs suppress gene expression post-transcriptionally [1]. DNA methylation contributes to natural human variation [2]. Epigenetic changes in genes associated with age affect life expectancy and longevity [3]. Altered DNA methylation patterns may account for phenotypic changes associated with human aging, neurodegeneration and cancer [4-6].

Dramatic changes in epigenetic events are common in pathogenic genes associated with major problems of health and particularly in cancer. These genes and their products are fundamental targets for an efficient anti-tumoral treatment and for biomarkers of diagnosis and prognosis [7]. The impact of epigenetic events in oncogenic genes has been documented in many different types of cancer.

Epigenetic inactivation of multiple tumor suppressor genes plays an important role in the tumorigenesis of colorectal carcinoma (CRC) [8]. Changes in DNA methylation, whether hypo or hypermethylation, have been shown to be associated with the progression of CRC. Methylation changes substantially in the progression from normal mucosa to adenoma and to carcinoma [9]. Hypermethylation of several gene clusters has been termed CpG island methylator phenotype and appears to define a subgroup of colon cancer distinctly characterized by pathological, clinical, and molecular features. DNA methylation of multiple promoters may serve as a biomarker for early detection in stool and blood DNA and as a tool for monitoring patients with CRC. DNA methylation patterns may also be predictors of metastatic or aggressive CRC [10]. Nasopharyngeal carcinoma associated gene 6 (NGX6) is down-regulated in most colon cancer cell lines and tumor tissues. The sequence spanning positions -157 to +276 was identified as the NGX6 promoter, in which no canonical TATA boxes were found, while two CAAT boxes and GC boxes were discovered. Dense methylation of the NGX6 promoter was associated with CRC and metastasis [11]. Serrated adenocarcinoma is a subset of CRC, which accounts for about 10% of all CRCs and follows an alternative pathway in which serrated polyps replace the traditional adenoma as the precursor lesion to CRC. Serrated polyps form a heterogeneous group of colorectal lesions that includes hyperplastic polyps, sessile serrated adenoma, traditional serrated adenoma and mixed polyps. Serrated polyps exhibited a distinct genetic pattern, with KRAS and BRAF having an important contribution to its development, and also show microsatellite instability and the CpG island methylator phenotype [12]. Several miRNAs are already known to be dysregulated in CRCs and have been linked to biological processes involved in tumor progression and

response to anti-cancer therapies (oxaliplatin, irinotecan, cetuximab, panitumumab, bevacizumab, aflibercept and regorafenib) [13]. In two clinical trial cohorts, a systematic biomarker discovery and validation approach identified miR-320e to be a novel prognostic biomarker that is associated with adverse clinical outcome in stage III CRC patients treated with 5-FU-based adjuvant chemotherapy [14].

CRC with microsatellite instability (MSI) displays unique clinic pathologic features including a mucinous pattern with frequent expression of the secreted mucins MUC2 and MUC5AC. MUC2 and MUC5AC are frequently hypomethylated in CRC. MUC2 and MUC5AC hypomethylation was associated with MUC2 and MUC5AC protein expression, poor differentiation and microsatellite instability (MSI) status. MUC5AC hypomethylation was specific to MSI cancers, and it was associated with BRAF mutation and CpG island methylator phenotype [15].

Metastasis is responsible for most cancer-related deaths and epigenetic changes contribute to the dissemination process. In melanoma, a hypomethylation event that reactivates a cryptic transcript of the Rab GTPase activating protein TBC1D16 was found to be a characteristic feature of the metastatic cascade. This short isoform of TBC1D16 exacerbates melanoma growth and metastasis both in vitro and in vivo. RAB5C is a new TBC1D16 target that regulates EGFR in melanoma cells. Epigenetic reactivation of TBC1D16-47KD is associated with poor clinical outcome, while conferring greater sensitivity to BRAF and MEK inhibitors [16].

The analysis of 409 cancer-related genes in HER2-positive breast cancer tissue samples revealed resistance to trastuzumab. The WNT pathway was potentially activated by aberrant methylation of its negative regulators, such as DKK3 and SFRP1, in 9 breast cancers. The AKT/mTOR pathway was activated by mutations of PIK3CA in 5 breast cancers. The Notch pathway was potentially activated by mutations of NOTCH1 and NOTCH2 in 4 breast cancers. The p53 pathway was inactivated by mutations of TP53 in 13 breast cancers and potentially by aberrant methylation of its downstream genes in 10 breast cancers. Cell adhesion was affected by mutations of CDH1 in 1 breast cancer [17].

ANXA1 is overexpressed in familial breast cancer (BC) patients with BRCA1/2 mutations and this correlates with poor prognosis features: triple negative and poorly differentiated tumors. ANXA1 might be a biomarker candidate for breast cancer survival prediction

*Corresponding author: Ramón Cacabelos, EuroEspes Biomedical Research Center, Institute of Medical Science and Genomic Medicine, 15165-Bergondo, Corunna, Spain, Tel: +34-981-780505; E-mail: rcacabelos@euroespes.com

in high risk groups such as HER2^{+} cases [18]. The steroid receptor coactivator SRC3 is essential for the transcriptional activity of estrogen receptor α. BC pathogenesis relies upon SRC3, which also has been implicated in endocrine resistance. SRC3 is post-translationally modified by phosphorylation. While total SRC3 is selectively found at enhancer regions, SRC3-pS543 is recruited to promoters of ERα responsive genes. SRC3-pS543 was associated with improved survival. Phosphorylation of SRC3 at S543 affects its genomic interactions on a genome-wide level, where SRC3-pS543 is selectively recruited to promoters of ERα-responsive genes. SRC3-pS543 is a prognostic marker, and a predictive marker of response to endocrine therapy [19].

HOXC6 is a homeobox-containing gene which is overexpressed in a variety of cancers including breast and prostate cancers. E2 induces HOXC6 expression in BC cells. HOXC6 expression is also induced upon exposure to BPA. Estrogen-receptor-alpha (ERα) and ER-coregulators such as MLL-histone methylases are bound to the HOXC6 promoter upon exposure to E2 or BPA, resulting in increased histone H3K4-trimethylation, histone acetylation, and recruitment of RNA polymerase II at the HOXC6 promoter. HOXC6 overexpression induces expression of tumor growth factors and facilitates growth 3D-colony formation, indicating its potential roles in tumor growth [20].

The expression of protein tyrosine phosphatase PTPN12 is epigenetically regulated, and 5-Azacytidine (5-Azac) modifies the expression of PTPN12 in the MDA-MB-231 and BT-549 triple-negative breast cancer cell lines [21].

Gadd45g (growth arrest and DNA-damage-inducible 45 gamma) is a stress-response protein, which has been implicated in several biological processes, including DNA repair, the cell cycle and cell differentiation. MiR-383 is a negative regulator of Gadd45g. Forced expression of miR-383 decreases the expression of Gadd45g through binding to the 3' untranslated region (3'-UTR), whereas inhibition of miR-383 increases Gadd45g expression. The presence of miR-383 increased the cellular sensitivity to DNA damage in breast cancer cells, which is rescued by ectopic expression of Gadd45g without the 3'-UTR [22].

Gene expression profiles and data of gene promoter methylation for a large panel of non-small cell lung cancer cell lines identified 578 candidate genes with expression levels that were inversely correlated to the degree of DNA methylation. These candidate genes were differentially methylated in normal lung tissue versus non-small cell lung cancer tumors, and segregated by histologic and tumor subtypes. Genes related to the epithelial-to-mesenchymal transition, such as AXL, ESRP1, HoxB4, and SPINT1/2, were among the nearly 20% of the candidate genes that were differentially methylated between epithelial and mesenchymal cells. Greater numbers of genes were methylated in the mesenchymal cells and their expressions were upregulated by 5-azacytidine treatment. Methylation of the candidate genes was associated with erlotinib resistance in wild-type EGFR cell lines [23].

PRDM2, PRDM5, PRDM16 promoters are methylated and their expression is suppressed in lung cancer cells. Demethylation drug 5-aza-2dC could upregulate the expression of PRDM2, PRDM5, PRDM16 and suppress lung cancer cell growth [24].

KCNJ2/Kir2.1, a member of the classical inwardly rectifying potassium channel family, is commonly expressed in a wide range of tissues and cell types. Kir2.1 is associated with small-cell lung cancer (SCLC) multidrug resistance (MDR). KCNJ2/Kir2.1 was expressed in 44.23% (23/52) of SCLC tissues. Overexpression of KCNJ2/Kir2.1 was correlated with the clinical stage and chemotherapy response in SCLC patients. Knockdown of KCNJ2/Kir2.1 expression using KCNJ2/Kir2.1 shRNA in H69AR and H446AR cells inhibited cell growth and

sensitized the cancer cells to chemotherapeutic drugs by increasing cell apoptosis and cell cycle arrest. Multidrug resistance protein 1 (MRP1/ABCC1) interacts with KCNJ2/Kir2.1. KCNJ2/Kir2.1 modulates cell growth and drug resistance by regulating MRP1/ABCC1 expression and is simultaneously regulated by the Ras/MAPK pathway and miR-7 [25].

Glutathione S-transferase 1 (GSTP1) inactivation is associated with CpG island promoter hypermethylation in prostate cancer (PC). Plasma mGSTP1 DNA is a potential prognostic marker in men with PC as well as a potential surrogate therapeutic efficacy marker for chemotherapy [26].

The RUNX gene family is involved in hematological malignancies. Epigenetic studies to elucidate RUNX inactivation in leukemia cell lines and samples from acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL) and myelodysplastic syndrome (MDS) patients revealed that promoter DNA methylation of RUNX1, 2, and 3 in 23 RUNX1 and RUNX2 gene promoters were mostly unmethylated in cell lines and clinical samples. Hypermethylation of RUNX3 was frequent among cell lines (74%) and highly variable among patient samples, with clear association with cytogenetic status. High frequency of RUNX3 hypermethylation (85%) was found in AML patients with inv(16)(p13.1q22) compared to other AML subtypes (31%). RUNX3 hypermethylation was also frequent in ALL (100%) but low in MDS (21%). Hypermethylation of RUNX3 was correlated with low levels of protein, and treatment of cell lines with the DNA demethylating agent, decitabine, resulted in mRNA re-expression. Relapse-free survival of non-inv(16)(p13.1q22) AML patients without RUNX3 methylation was better than that of methylated cases. RUNX3 silencing is an important event in inv(16)(p13.1q22) leukemias [27].

miRNAs located at chromosome fragile sites play important roles in regulating critical genes associated with myeloma pathogenesis, disease progression and drug resistance. MiR-33b (chromosome 17p) is one of the dysregulated miRNAs in the sera of newly-diagnosed multiple myeloma (MM) patients. The expression of miR-33b is down-regulated in newly-diagnosed and relapsed MM patients compared to remission patients and health donors. Patients with del (13q), del (17p),t(4;14) and high-risk genetic abnormalities have lower expression levels of miR-33b compared to patients without those abnormalities. Patients with miR-33b low expression have shortened survival and might be associated with drug resistance to bortezomib-based treatment [28].

These are but a few examples of the vast repertoire that epigenetic biomarkers may provide for the clinical management of oncogenic processes. Furthermore, some modalities of cancer are susceptible to epigenetic therapy with epigenetic drugs, since epigenetic modifications are reversible and can be targeted by pharmacological intervention [6].

References

1. Szulwach KE, Jin P (2014) Integrating DNA methylation dynamics into a framework for understanding epigenetic codes. Bioessays 36: 107-117.

2. Heyn H, Moran S, Hernando-Herraez I, Sayols S, Gomez A, et al. (2013) DNA methylation contributes to natural human variation. Genome Res 23: 1363-1372.

3. Ben-Avraham D (2015) Epigenetics of aging. Adv Exp Med Biol 847: 179-191.

4. Jones MJ, Goodman SJ, Kobor MS (2015) DNA methylation and healthy human aging. Aging Cell (Published online 25 April 2015).

5. Cacabelos R, Torrellas C (2014) Epigenetic drug discovery for Alzheimer's disease. Expert Opin Drug Discov 9: 1059-1086.

6. Cacabelos R (2014) Epigenomic networking in drug development: from pathogenic mechanisms to pharmacogenomics. Drug Dev Res 75: 348-365.

7. Coppedè F, Lopomo A, Spisni R, Migliore L (2014) Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. World J Gastroenterol 20: 943-956.

8. Sakai E, Nakajima A, Kaneda A (2014) Accumulation of aberrant DNA methylation during colorectal cancer development. World J Gastroenterol 20: 978-987.

9. Beggs AD, Jones A, El-Bahrawy M, Abulafi M, Hogdson SV, et al. (2013) Whole-genome methylation analysis of benign and malignant colorectal tumors. J Pathol 229: 697-704.

10. Kim JS, Lee SC, Min HY, Park KH, Hyun SY, et al. (2015) Activation of insulin-like growth factor receptor signaling mediates resistance to histone deacetylase inhibitors. Cancer Lett 361: 197-206.

11. Liu M, Peng Y, Wang X, Guo Q, Shen S, et al. (2010) NGX6 gene mediated by promoter methylation as a potential molecular marker in colorectal cancer. BMC Cancer (Published online 27 April 2010).

12. Yamane L, Scapulatempo-Neto C, Reis RM, Guimarães DP (2014) Serrated pathway in colorectal carcinogenesis. World J Gastroenterol 20: 2634-2640.

13. Stiegelbauer V, Perakis S, Deutsch A, et al. (2014) MicroRNAs as novel predictive biomarkers and therapeutic targets in colorectal cancer. World J Gastroenterol 20: 11727-11735.

14. Perez-Carbonell L, Sinicrope FA, Alberts SR, Oberg AL, Balaquer F, et al. (2015) MiR-320e is a novel prognostic biomarker in colorectal cancer. Br J Cancer 113: 83-90.

15. Renaud F, Vincent A, Mariette C, Crepin M, Copin MC, et al. (2015) MUC5AC hypomethylation is a predictor of microsatellite instability independently of clinical factors associated with colorectal cancer. Int J Cancer 136: 2811-2821.

16. Vizoso M, Ferreira HJ, Lopez-Serra P, Carmona FJ, Martinez-Cardus A et al. (2015) Epigenetic activation of a cryptic TBC1D16 transcript enhances melanoma progression by targeting EGFR. Nat Med 21: 741-750.

17. Yamaguchi T, Mukai H, Yamashita S, Fujii S, Ushijima T (2015) Comprehensive DNA Methylation and Extensive Mutation Analyses of HER2-Positive Breast Cancer. Oncology 88: 377-384.

18. Sobral-Leite M, Wesseling J, Smit VT, Nevanlinna H, Miltenburg MHV, et al. (2015) Annexin A1 expression in a pooled breast cancer series: association with tumor subtypes and prognosis. BMC Med (Published online 2 July 2015).

19. Zwart W, Flach KD, Rudraraju B, Abdel- Fatah TM, Gojis O, et al. (2015) SRC3 phosphorylation at Serine 543 is a positive independent prognostic factor in ER positive breast cancer. Clin Cancer Res (Published online 14 September 2015).

20. Hussain I, Bhan A, Ansari KI, Deb P, Bobzean SA, et al. (2015) Bisphenol-A induces expression of HOXC6, an estrogen-regulated homeobox-containing gene associated with breast cancer. Biochim Biophys Acta 1849: 697-708.

21. Thummuri D, Kumar S, Surapaneni SK, Tikoo K (2015) Epigenetic regulation of protein tyrosine phosphatase PTPN12 in triple-negative breast cancer. Life Sci 130: 73-80.

22. Zhao L, Gu H, Chang J, Wu J, Wang D, et al. (2014) MicroRNA-383 regulates the apoptosis of tumor cells through targeting Gadd45g. PLoS One (Published online 21 November 2014).

23. Lin SH, Wang J, Saintigny P, Chia-Chin W, Giri U, et al. (2014) Genes suppressed by DNA methylation in non-small cell lung cancer reveal the epigenetics of epithelial-mesenchymal transition. BMC Genomics (Published online 8 December 2014).

24. Tan SX, Hu RC, Liu JJ, Tan YL, Liu WE, et al. (2014) Methylation of PRDM2, PRDM5 and PRDM16 genes in lung cancer cells. Int J Clin Exp Pathol 7: 2305-2311.

25. Liu H, Huang J, Peng J, Wu X, Zhang Y, et al. (2015) Up regulation of the inwardly rectifying potassium channel Kir2.1 (KCNJ2) modulates multidrug resistance of small-cell lung cancer under the regulation of miR-7 and the Ras/MAPK pathway. Mol Cancer (Published online 12 March 2015).

26. Mahon KL, Qu W, Devaney J, Paul C, Castillo L, et al. (2014) Methylated Glutathione S-transferase 1 (mGSTP1) is a potential plasma free DNA epigenetic marker of prognosis and response to chemotherapy in castrate-resistant prostate cancer. Br J Cancer 111: 1802-1809.

27. Estécio MR, Maddipoti S, Bueso-Ramos C, DiNardo CD, Yang H, et al. (2015) RUNX3 promoter hypermethylation is frequent in leukaemia cell lines and associated with acute myeloid leukaemia inv(16) subtype. Br J Haematol 169: 344-351.

28. Li F, Hao M, Feng X, Zang M, Qin Y, et al. (2015) Down regulated miR-33b is a novel predictor associated with disease progression and poor prognosis in multiple myeloma. Leuk Res 39: 793-799.