Research Highlights

Highlights from the latest articles in epigenomics

Intertwined roles of methylation and the Epstein–Barr virus in the pathogenesis of nasopharyngeal carcinoma

Evaluation of: Loyo M, Brait M, Kim MS *et al.* A survey of methylated candidate tumor suppressor genes in nasopharyngeal carcinoma. *Int. J. Cancer* 128(6), 1393–1403 (2011).

The herpes virus, Epstein–Barr virus (EBV), the first identified human oncogenic virus, was discovered nearly 50 years ago, and exists in a latent stage in approximately 95% of humans [1–4]. Its large genome codes for multiple protein products and several mRNAs. It is the major causative agent of several lymphoma types as well as some epithelial cancers, notably nasopharyngeal carcinoma (NPC) and a subset of gastric carcinomas. In most cells the virus is usually in a latent form with only part of the protein program expressed. Viral transformation may result in increased global methylation of the host genome as well as methylation of specific genes, while the latency program in turn is controlled by host-mediated methylation of viral genes [2–4].

Nasopharyngeal carcinoma, a rare disease in most parts of the world, demonstrates considerable ethnic and geographic differences in incidence, being common in parts of Asia, especially Southern China [5]. Early diagnosis is associated with high cure rates. Most tumors lack squamous features. Almost all of the poorly differentiated tumors contain EBV, which appears early during pathogenesis. Loyo *et al.* compared the methylation pattern of 50 NPC tumors, all of them poorly differentiated, with that of

nonmalignant nasopharyngeal epithelium, using a quantitative methylation-specific PCR assay for 18 genes [6]. While methylation of three of these genes was known to be associated with NPC, the selection of the others was not explained. Methylation of several genes was associated with NPCs, and the combination of three of them had high specificity and sensitivity. Their findings may translate into a biomarker panel for early detection. However, several questions remain. Why wasn't a global methylation approach used on these rare tumors instead of a seemingly random selection? Why weren't several genes located in regions of high-allelic loss in NPC such as *INK4A* (chromosome 9p) and *RASSF1A* (chromosome 3p) tested? Do the methylated genes represent EBV induced changes or are they specific for NPC pathogenesis? How will the proposed panel perform in preinvasive lesions? Despite these unanswered questions, the positive aspects of this study does offer us hope for the future.

The complex reciprocal methylation relationship between the host and virus offers us a fascinating window into the pathogenesis of viral oncogenesis. A fuller understanding of the cell–virus interplay may translate into early diagnosis and improved management.

References

- 1 Young LS, Rickinson AB. Epstein–Barr virus: 40 years on. *Nat. Rev. Cancer* 4(10), 757–768 (2004).
- 2 Stein RA. Epigenetics – the link between infectious diseases and cancer. *JAMA* 305(14), 1484–1485 (2011).

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- 3 Shah KM, Young LS. Epstein–Barr virus and carcinogenesis: beyond Burkitt's lymphoma. *Clin. Microbiol. Infect.* 15(11), 982–988 (2009).
- 4 Ryan JL, Jones RJ, Kenney SC *et al.* Epstein–Barr virus-specific methylation of human genes in gastric cancer cells. *Infect. Agent. Cancer* 5, 27 (2010).
- 5 Wei WI, Sham JS. Nasopharyngeal carcinoma. *Lancet* 365(9476), 2041–2054 (2005).
- 6 Loyo M, Brait M, Kim MS *et al.* A survey of methylated candidate tumor suppressor genes in nasopharyngeal carcinoma. *Int. J. Cancer* 128(6), 1393–1403 (2011).

Methylation of homeobox genes in prostate and breast cancers

Evaluation of: Yamamoto M, Cid E, Bru S, Yamamoto F. Rare and frequent promoter methylation, respectively, of *TSHZ2* and 3 genes that are both downregulated in expression in breast and prostate cancers. *PLoS ONE* 6(3), e17149 (2011).

Prostate and breast cancers share certain attributes – they are initially hormone dependent, which offers opportunities for therapy, but become resistant at later stages [1]. Yamamoto and colleagues have studied silencing of homeobox genes in these cancers [2]. Homeobox genes play crucial roles in development and act as transcriptional modulators [3]. The authors combined specifically designed multiplex reverse transcription (RT)-PCR assay designated as systematic multiplex RT-PCR [4], real-time quantitative RT-PCR and genome-wide expression studies, and investigated gene-expression differences between

tumor cell lines and primary nonmalignant cultures. Results were confirmed by testing tumors. Of the 194 homeobox and related genes studied, more than 35 differentially expressed homeobox genes were identified by systematic multiplex RT-PCR. Of the 194 homeobox and related genes studied, the two top downregulated genes belonged to the three gene family of Teashirt genes, not previously implicated in carcinogenesis. Of great interest, one of these genes, *TSHZ2*, was downregulated by promoter methylation, while the related gene *TSHZ3* was largely unmethylated. Thus, two highly related genes, both with promoter CpG islands, are silenced in breast and prostate (and perhaps other) cancers by different mechanisms. There are multiple ways of inactivating tumor suppressor genes other than DNA methylation – copy number changes, inactivating mutations, histone modifications and remodeling, as well as other poorly understood mechanisms of transcriptional or translational controls.

The authors' work confirms that homeobox genes can act as tumor suppressor genes and that they can be inactivated by multiple mechanisms.

References

- 1 Risbridger GP, Davis ID, Birrell SN, Tilley WD. Breast and prostate cancer: more similar than different. *Nat. Rev. Cancer* 10(3), 205–212 (2010).
- 2 Yamamoto M, Cid E, Bru S, Yamamoto F. Rare and frequent promoter methylation, respectively, of *TSHZ2* and 3 genes that are both downregulated in expression in breast and prostate cancers. *PLoS ONE* 6(3), e17149 (2011).
- 3 McGinnis W, Krumlauf R. Homeobox genes and axial patterning. *Cell* 68(2), 283–302 (1992).
- 4 Yamamoto M, Metoki R, Yamamoto F. Systematic multiplex polymerase chain reaction and reverse transcription-polymerase chain reaction analyses of changes in copy number and expression of proto-oncogenes and tumor suppressor genes in cancer tissues and cell lines. *Electrophoresis* 25(20), 3349–3356 (2004).

Epigenetics and the prognosis of neuroblastoma

Evaluation of: Caren H, Djos A, Nethander M *et al.* Identification of epigenetically regulated genes that predict patient outcome in neuroblastoma. *BMC Cancer* 11, 66 (2011).

Neuroblastoma is a relatively common pediatric tumor. It is an embryonal tumor of the autonomic nervous system [1,2], and

is characterized by considerable heterogeneity of clinical and pathological features as well as outcome. As with many embryonal tumors, neuroblastoma has few characteristic genetic abnormalities, mainly copy number alterations, and most of the described ones are associated with tumor subsets and outcome. The epigenetics of neuroblastoma has also been reported in several publications, and has

been associated with outcome [3–6]. At least two of these reports have used genome-wide approaches [3,6]. Caren *et al.* also used a global approach [7], but their report may represent the most comprehensive approach to identifying neuroblastoma-associated methylated genes and relating them to patient outcome. They used global expression studies of four cell lines to identify genes pharmacologically

activated after exposure to a demethylating agent or a histone deacetylases inhibitor. Reactivation was confirmed with quantitative PCR assays. Because some of the genes so identified may represent secondary activation events rather than tumor-associated methylated genes, they combined their studies with global methylation studies of 59 tumors. They identified eight genes, most of them not previously associated with neuroblastoma. Differential methylation of three genes, and expression of two of them, was related to adverse outcome.

The ability to accurately predict outcome and response to therapy is crucial for the optimal management of patients with neuroblastoma. It will be important to determine if epigenetics can contribute

to genetic studies and clinicopathological features to form an algorithm for improved prognosis.

References

- 1 Maris JM. Recent advances in neuroblastoma. *N. Engl. J. Med.* 362, 2202–2211 (2010).
- 2 Park JR, Eggert A, Caron H. Neuroblastoma: biology, prognosis, and treatment. *Hematol. Oncol. Clin. North Am.* 24, 65–86 (2010).
- 3 Buckley PG, Das S, Bryan K *et al.* Genome-wide DNA methylation analysis of neuroblastic tumors reveals clinically relevant epigenetic events and large-scale epigenomic alterations localized to telomeric regions. *Int. J. Cancer* 128, 2296–2305 (2011).
- 4 Grau E, Oltra S, Martinez F *et al.* MAGE-A1 expression is associated with good prognosis

in neuroblastoma tumors. *J. Cancer Res. Clin. Oncol.* 135, 523–531 (2009).

- 5 Hoebeek J, Michels E, Pattyn F *et al.* Aberrant methylation of candidate tumor suppressor genes in neuroblastoma. *Cancer Lett.* 273, 336–346 (2009).
- 6 Ohira M, Nakagawara A. Global genomic and RNA profiles for novel risk stratification of neuroblastoma. *Cancer Sci.* 101, 2295–2301 (2010).
- 7 Caren H, Djos A, Nethander M *et al.* Identification of epigenetically regulated genes that predict patient outcome in neuroblastoma. *BMC Cancer* 11, 66 (2011).

Can epigenetics contribute to the early diagnosis of renal cell carcinoma?

Evaluation of: Morris MR, Ricketts CJ, Gentle D *et al.* Genome-wide methylation analysis identifies epigenetically inactivated candidate tumour suppressor genes in renal cell carcinoma. *Oncogene* 30(12), 1390–1401 (2011).

Renal cell carcinoma (RCC) arose in 58,000 patients in the USA in 2010 and approximately 13,000 died from their disease [1]. RCC tumors often are asymptomatic and patients that present with advanced disease have a 2-year survival rate of only 18% [2]. The development of new early-detection methods and therapy is therefore urgently needed. In the past decade, several important tumor suppressor genes in RCC were found inactivated by epigenetic promoter hypermethylation, including *RASSF1A* [3], *VHL* [4] and *SFRP1* [5]. These findings indicated that the additional identification of DNA methylation-inactivated tumor suppressor genes may provide new early detection biomarkers and therapeutic targets for RCC.

The recent development and application of multiple genome-wide methylation technologies such as methylation DNA immunoprecipitation (MeDIP) and Illumina methylation microarray offer the promise to efficiently identify additional hypermethylation-silenced tumor suppressor genes in cancers. Morris *et al.* combined genome-wide methylation MeDIP and human promoter array (MeDIP-chip) and the genome-wide functional epigenetic expression array with demethylating agent 5-aza-2'-deoxycytidine [6]. They screened cell lines and a few tumors, and validated their findings in a larger tumor test set. Several genes were identified as being differentially methylated in tumors, and RNAi knockdown studies indicated that several of the genes regulate tumor growth and that *SCUBE3* methylation is an adverse prognostic factor. These findings are of interest, but need to be translated into patient management. One method would be to determine whether screening urine for a panel of methylated genes is an aid for early diagnosis.

References

- 1 Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J. Clin.* 60(5), 277–300 (2010).
- 2 Linehan WM, Pinto PA, Srinivasan R *et al.* Identification of the genes for kidney cancer: opportunity for disease-specific targeted therapeutics. *Clin. Cancer Res.* 13(2 Pt 2), 671S–679S (2007).
- 3 Morrissey C, Martinez A, Zatyka M *et al.* Epigenetic inactivation of the *RASSF1A* 3p21.3 tumor suppressor gene in both clear cell and papillary renal cell carcinoma. *Cancer Res.* 61(19), 7277–7281 (2001).
- 4 Linehan WM, Rubin JS, Bottaro DP. *VHL* loss of function and its impact on oncogenic signaling networks in clear cell renal cell carcinoma. *Int. J. Biochem. Cell Biol.* 41(4), 753–756 (2009).
- 5 Dahl E, Wiesmann F, Woenckhaus M *et al.* Frequent loss of *SFRP1* expression in multiple human solid tumours: association with aberrant promoter methylation in renal cell carcinoma. *Oncogene* 26(38), 5680–5691 (2007).
- 6 Morris MR, Ricketts CJ, Gentle D *et al.* Genome-wide methylation analysis identifies epigenetically inactivated candidate tumour suppressor genes in renal cell carcinoma. *Oncogene* 30(12), 1390–1401 (2011).