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## Mucins: a new family of epigenetic biomarkers in epithelial cancers

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# Expert Opinion

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# Expert Opinion

1. Introduction
2. Mucins and epigenetics
3. Expert opinion

## Mucins: a new family of epigenetic biomarkers in epithelial cancers

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**Background:** Epigenetic regulation of gene expression is a common feature of cancer development and progression. The search for new biomarkers and tools to detect cancer in its early stages has unveiled the usefulness of epigenetics and genes epigenetically regulated as potential targets. Among them, genes encoding mucins has been shown to be regulated by DNA methylation and histone modifications in epithelial cancer cells. These genes encode either secreted glycoproteins necessary for epithelial homeostasis or membrane-bound glycoproteins that participate in tumor progression. **Objective:** The important biological functions played by these large molecules in pathophysiology of the epithelia make them key genes to target to propose new therapeutic strategies and new diagnostic and/or prognostic tools in cancer. **Results:** In that context, the recent data regarding the epigenetic regulation of these genes are reported and their potential as biomarkers in cancer is discussed. Mucin genes are also potentially interesting to study as they may be regulated by miRNAs but also regulate miRNA activity. **Conclusion:** Epigenetic regulation of mucin genes is at its dawn, but there is great potential in that research to (with new technologies and high-throughput methods) provide quickly new biomarkers (diagnostic and/or prognostic), help tumor identification/classification and propose new therapeutic targets to the clinician and pathologist.

**Keywords:** biomarker, cancer, epigenetics, mucin

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### 1. Introduction

The term 'epigenetic' refers to a heritable change in the pattern of gene expression that is driven by mechanisms other than alterations in the primary nucleotide sequence of the gene [1,2]. Epigenetic changes in the genome include DNA methylation and histone modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation), two mechanisms that are often tightly linked in the regulation of gene expression and involved in many cellular processes [3]. For example, DNA methylation is a crucial epigenetic modification that drives embryonic development, X-chromosome inactivation, genomic imprinting and chromosome stability, whereas histone modifications govern rearrangement of chromatin structure.

DNA methylation occurs on CpG dinucleotides and follows a cell-specific profile that is established during development by DNA methyltransferases DNMT3A and DNMT3B [4] and is maintained through cell division by DNMT1 [5]. In the human genome, CpG sites are statistically underrepresented but are clustered in GC-rich regions termed CpG islands. These CpG islands are often associated with promoter regions or first exons of half the genes in our genome. In normal cells, 80% of CpG dinucleotides that are not associated with

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55 CpG islands (predominantly in intergenic and intronic  
regions of DNA, repeat sequences and transposable elements)  
are heavily methylated, whereas CpG islands are usually  
60 unmethylated, whether or not the gene is transcriptionally  
active [6]. On the contrary, the cancer genome is frequently  
characterized by both global hypomethylation of intergenic  
regions resulting in chromosomal instability [5] and specific  
hypermethylation of CpG islands in promoters resulting in  
aberrantly silenced tumor suppressor genes [6].

65 DNA methylation by itself does not directly repress  
transcription. The process of silencing needs the involvement  
of the epigenetic machinery composed of chromatin modi-  
fier enzymes, such as histone deacetylases (HDACs) and  
histone methyltransferases (HMTs), and proteins that are  
70 able to read methylation marks, namely the methylCpG  
binding proteins (MBPs). MBPs recognize methylated DNA  
and recruit multiprotein complexes containing HDACs that  
remove acetyl residues from protruding histone tails, hence  
tightly compacting chromatin and suppressing promoter  
access to transcription machinery [7].

75 In the collaboration between the two epigenetic mechanisms  
that silences gene expression in cancer it is unclear at the  
moment whether DNA methylation or histone modification  
is dominant. Studies using drugs that inhibit epigenetic  
mechanisms showed that HDAC inhibitors alone cannot  
80 induce the expression of hypermethylated genes, indicating  
that DNA methylation may be preponderant. However, in  
some cases, histone (de)acetylation can drive DNA (de)  
methylation [8,9], suggesting that DNA methylation may  
simply be a marker of silent chromatin. Nevertheless, stable  
85 gene repression by epigenetics in cancers seems to involve  
both mechanisms and appears to be a specific mark of cancer  
cells. The recent discovery of small non-protein-coding RNA,  
named micro-RNAs (miRNAs), which target 3'-untranslated  
regions (3'-UTR) of genes and repress either their maturation  
90 or lead them to their degradation, added a new layer to  
epigenetic regulation by means of RNA interference [10-12].

As altered patterns of epigenetic modifications are observed  
in the early stage of carcinogenesis, it was suggested several  
95 years ago that aberrant DNA methylation may be used as a  
biological tool for early detection and prognosis of cancer [13-15].  
Moreover, the potential reversibility of epigenetic modifications  
gives new opportunities for the clinical treatment of cancer [16].  
Thus, identifying new pharmacological inhibitors of epigenetic  
100 modifications has become the focus of research in many  
laboratories, and both DNMT and HDAC inhibitors have  
shown promising results in clinical trials [17,18].

Next to pharmacological inhibitors, growing interest has  
been shown by researchers in controlling gene expression  
during the early steps of carcinogenesis and/or using altered  
105 pattern of expression of these genes as diagnostic/prognostic  
markers. Epigenetically marked genes offer great and exciting  
possibilities in that sense because methylation occurs during  
the early stages of cancer. Among them, genes encoding  
secreted and membrane-bound mucins are often aberrantly  
109

expressed in tumors and this finding has led to the hypothe- 110  
sis that mucins may be used as diagnostic and/or prognos-  
tic factors in epithelial cancers of different origins [19-22].  
Alterations of expression include both silencing and overex-  
pression of mucin genes while their promoters share a GC-rich  
115 structure and, for most of them, contain a CpG island [20].  
Moreover, the four genes *MUC2*, *MUC5AC*, *MUC5B* and  
*MUC6*, encoding the gel-forming mucins, are clustered on  
the p15.5 region of chromosome 11 (11p15 MUC genes) [23],  
in an area known to be a hot spot of abnormal methy- 120  
lation in cancer [24]. This explains why most of the studies  
regarding epigenetic regulation of mucin genes focused on  
the 11p15 MUC cluster.

In this paper, the studies that were conducted by the  
authors' laboratory are reviewed, as well as others about the  
epigenetic regulation of the 11p15 MUC genes and those 125  
about *MUC1* and *MUC4* genes that encode two membrane-  
bound mucins known to participate in tumor progression  
and alter cancer cell biological properties [25-27]. The second  
part of the review opens up to the recent development of  
new technologies to study epigenetic regulation, and the 130  
exciting new avenues that mucin gene regulation by miRNAs  
may provide to the clinicians in terms of therapeutic tools  
are discussed. No data exist about mucin gene regulation by  
miRNAs in the literature, so for the first time miRNA data- 135  
bases were searched and the (long) lists of potential regulatory  
miRNAs targeting 3'-UTRs of mucin genes established. The  
paper concludes with a discussion of what the detection of  
mucin gene methylation could bring to the clinician and/or  
pathologist as a diagnostic/prognostic tool for early detection  
140 of cancer and tumor identification/classification.

## 2. Mucins and epigenetics

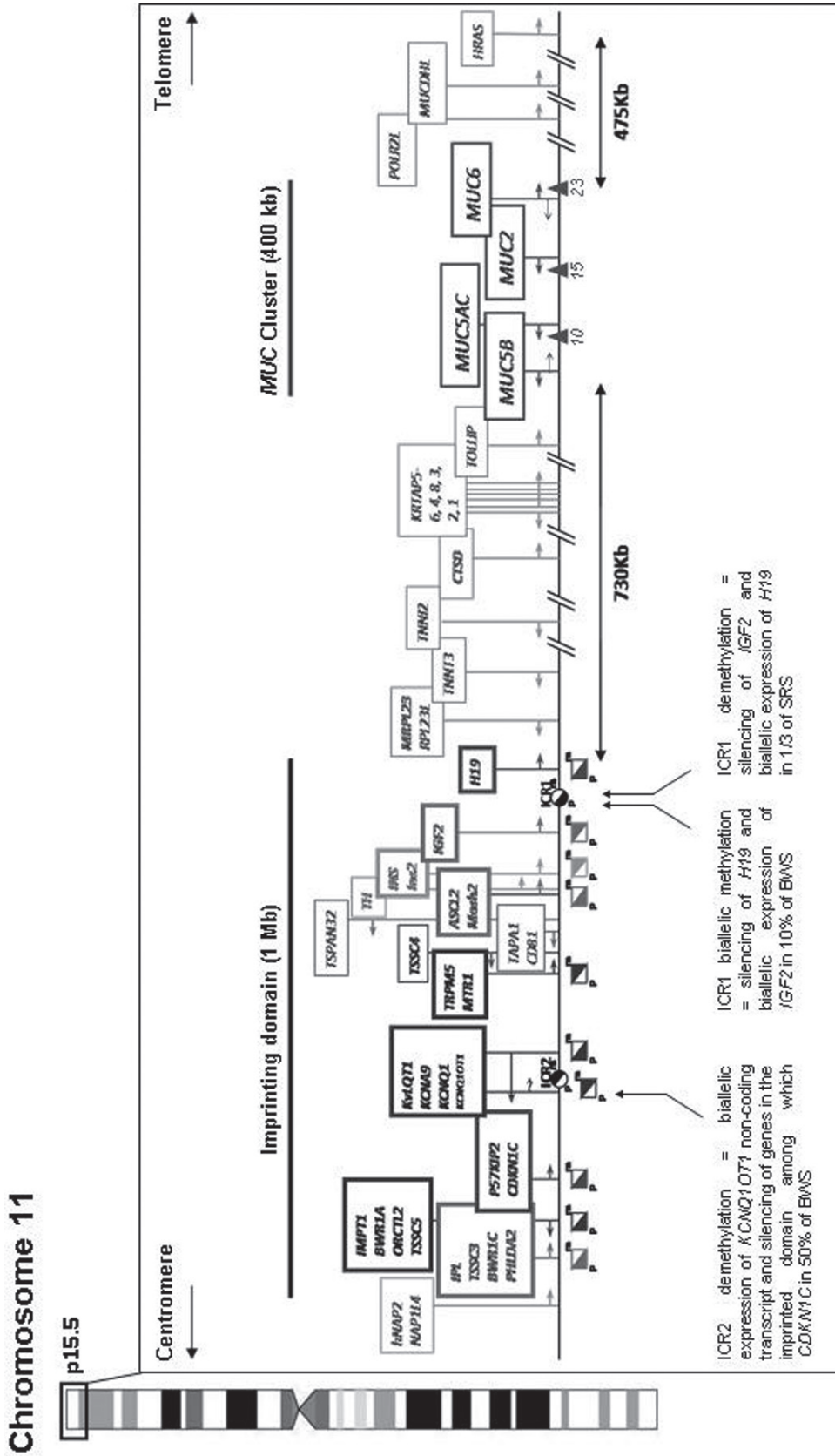
### 2.1 DNA methylation and histone marks

#### 2.1.1 The 11p15 mucin genes

##### 2.1.1.1 The 11p15.5 chromosomal region

The 11p15 chromosomal region is of particular interest for  
studying epigenetic mechanisms of regulation. Aberrant  
DNA methylation in this region is associated with severe  
developmental abnormalities [28,29] and cancer [24,30]. This 150  
chromosomal region is characterized by one of the highest  
concentration of CpG islands in the genome, being separated  
from each other by 15 – 100 kb (Figure 1) [31]. Within this  
region lies a 1 Mb imprinted domain, containing several  
155 genes involved in growth and development, and potential  
tumor suppressor genes that are aberrantly methylated in  
cancer [24,32-34]. Interestingly, *MUC2*, one of the 11p15  
mucins, was recently proposed to have tumor suppressor activity in  
colorectal cancer [35].

The 11p15 MUC cluster is 400 kb in length and is situated 160  
~ 700 kb from the imprinted domain, between insulin-like  
growth factor-2 (*IGF-2*) and Harvey rat sarcoma viral oncogene  
homolog (*H-RAS*) genes, within a region containing 15 CpG  
islands (Figure 1) [23]. Within the cluster, mucin genes are 164



**Figure 1. The 11p15 chromosomal region.** Positions of the imprinting domain, the two imprinting control regions (ICR) and the mucin gene cluster (MUC) are indicated (black boxes). Full arrows indicate the transcription orientation for each gene. Dashed arrows indicate an antisense transcript (MUC5B, MUC6). Imprinted genes are boxed in gray and bold. Other known designations for each gene are indicated in the same box. Allele-specific methylation of imprinted genes is indicated by half-filled rectangles, p: paternal allele, m: maternal allele. Epigenetic abnormalities frequently described in Beckwith-Wiedemann Syndrome (BWS) and Silver-Russell Syndrome (SRS) are indicated. Triangles represent putative target regions for post-transcriptional regulation of mucin transcripts by miRNAs (MUC2, MUC5AC, MUC6). The number of putative miRNAs targeting these regions is indicated and is detailed in **Table 2**.



165 organized as follows, *MUC6–MUC2–MUC5AC–MUC5B*  
 from telomere to centromere, *MUC2*, *MUC5AC* and *MUC5B*  
 being transcribed from telomere to centromere, whereas *MUC6*  
 is transcribed in the opposite direction (Figure 1).

170 2.1.1.2 Epigenetic regulation of the 11p15 mucin genes

The first studies that aimed at studying epigenetic regulation  
 of mucin genes focused on *MUC2*. Based on the observation  
 that *MUC2* mucin expression was repressed in non-mucinous  
 colorectal cancers and their metastasis, Hanski and collaborators  
 were the first to investigate the role of DNA methylation in  
 175 *MUC2* silencing [36]. To this end, they used the methylation-  
 sensitive enzyme HpaII and the methylation-independent  
 enzyme MspI to digest DNA from colon carcinoma cell lines  
 expressing variable levels of *MUC2*, followed by Southern  
 blotting. They showed hypermethylation of *MUC2* promoter  
 in non-mucinous carcinoma cell lines [36,37].

Thereafter, arising bisulfite-treated genomic DNA-based  
 methods allowed more precise analysis of methylation profiles  
 of CpG sites involved in *MUC2* silencing. These studies  
 showed the involvement of 9 CpG sites situated directly  
 185 upstream of the transcriptional start site of *MUC2*, at -289,  
 -274, -246, -192, -160, -126, -118, -6 and -2, in the silencing  
 of the gene in non-expressing normal columnar cells and in  
 the non-mucinous carcinoma tissues of the colon [37] as well  
 as in pancreatic cancer cell lines [38]. The use of methylation-  
 sensitive single nucleotide primer extension (Ms-SNuPE)  
 allowed Mesquita and co-workers to demonstrate the role of  
 site-specific promoter hypomethylation in aberrant *MUC2*  
 mucin expression in mucinous gastric carcinoma [39].

In 2005, mapping of the methylation status of the 59 CpG  
 sites of *MUC2* promoter lying from position -1989 to position  
 +288 in pancreatic cancer cell lines confirmed the role of  
 site-specific methylation in *MUC2* silencing, and authors  
 described methylation of DNA binding sites for AP-2 and  
 Sp1 transcription factors. However, the authors did not  
 200 study the influence of methylation on *MUC2* activation by  
 these two transcription factors [40]. They also showed that  
 histone H3 modification status at *MUC2* promoter, and  
 especially demethylation of lysine 4, methylation of lysines 9  
 and 27 and deacetylation of lysines 9 and 27, tightly related  
 205 to DNA methylation, played an important role in *MUC2*  
 gene silencing [41].

Very few studies have been conducted concerning the  
 three other genes of the cluster. The first study concerning  
 epigenetic regulation of *MUC5B* was carried out at the  
 authors' laboratory and showed, using HpaII and MspI  
 enzymes combined with Southern blotting and cell treatment  
 with DNA methylation inhibitor 5-aza-22deoxycytidine  
 (5-aza), that the -1095/-695 region of *MUC5B* proximal  
 215 promoter was hypermethylated in gastric *MUC5B*-expressing  
 KATO-III cells, whereas methylation occurred throughout  
 the 55-flanking region in gastric low-expressing AGS  
 cells [20,42]. This pattern of methylation corresponded to the  
 219 downregulation of *MUC5B* apomucin expression observed

in undifferentiated gastric carcinomas [42]. The only study 220  
 carried out on *MUC5AC* and more particularly on one  
 CpG site in pancreatic cancer cell lines did not show any  
 correlation between epigenetic regulation of the gene and  
 expression of the apomucin [43].

To assess whether epigenetic regulation of the four 11p15 MUC 225  
 genes was concerted and associated with cancer, a simultaneous  
 study was undertaken of the four genes in a wide panel of  
 human epithelial cancer cell lines with different epithelial  
 origins and different status of differentiation [44]. The analysis  
 was carried out on large portions of the promoters, that is, 230  
 3.3 kb for *MUC2*, 1.3 kb for *MUC5AC*, 2.3 kb for *MUC5B*  
 and 1.8 kb for *MUC6*. *In silico* analysis showed that *MUC2*,  
*MUC5B* and *MUC6* promoters were particularly rich in  
 G+C nucleotides, up to 70, 80 and 75%, respectively, and  
 contained CpG islands of 115, 104 and 166 bp in length, 235  
 respectively, situated at -4647/-4533, -976/-873 and -160/+6,  
 whereas relatively few CpG sites were found in *MUC5AC* [44].

The studies indicate that regulation by methylation (after  
 treatment with 5-aza) and by histone acetylation (after  
 treatment with HDAC inhibitor trichostatin A [TSA]) is 240  
 both gene- and cell-specific. In some cases, TSA induced  
 repression of mucin gene expression, especially in confluent  
 differentiated cells expressing high levels of the mucin gene  
 of interest [44]. The same inhibitory mechanism by the  
 HDAC inhibitor sodium butyrate had previously been 245  
 found in *MUC2* high-expressing intestinal cancer cell  
 lines [45]. This may reflect a mechanism of definitive  
 function of the secretory lineage in order to maintain  
 epithelial homeostasis [46].

Bisulfite-treated genomic DNA sequencing and chromatin 250  
 immunoprecipitation (ChIP) assays confirmed that *MUC2*  
 was regulated by site-specific DNA methylation associated  
 with establishment of a repressive histone code, including  
 histone H3 deacetylation and methylation of lysine 9. Moreover,  
 our study covering the -3269/-1791 region of *MUC2* promoter 255  
 allowed us to identify 4 more relevant CpG sites at -3269,  
 -3199, -2331 and -1912. It was also shown that hyperm-  
 ethylation of *MUC5B* promoter was the main mechanism  
 responsible for its silencing in epithelial cancer cells and  
 involved a wide region of its distal promoter at -2677/-2163, as 260  
 well as cytosines at -434 and -421 in the proximal promoter.  
 Thus, we and others have identified key methylated cyto-  
 sines in *MUC2* and *MUC5B* promoters that should provide  
 useful tools, which are easy to detect, to screen mucin gene  
 repression by methylation in epithelial cancers and identify 265  
 tumors in which their repression may indicate good  
 or poor prognosis.

On the contrary, the expression of *MUC5AC* was rarely  
 influenced by epigenetic mechanisms and it was not possible  
 to identify CpG sites in its promoter which hypermethylation 270  
 would be associated with its silencing. Hypermethylation  
 of *MUC6* promoter was not correlated to its repression  
 because DNMT and HDAC inhibitors had no influence on  
 its expression in the cancer cell lines studied. Thus, epigenetic 274

275 regulation of 11p15 MUC genes is not concerted and  
involves gene-specific mechanisms [44].

280 Interestingly, our study also indicates that 11p15 mucin  
genes share a common feature regarding the methylation  
pattern of their promoters, which is that their CpG islands  
are heavily methylated regardless of their level of expression  
in the cancer cells studied [44]. However, these results will  
have to be confirmed in normal tissues compared with  
tumors because it is known that cancer cell lines often harbor  
an altered methylation pattern of their genome and excessive  
CpG island hypermethylation [47].

285 DNMT1, by a small interfering RNA (siRNA) approach,  
was identified as a potent regulator of *MUC2* and *MUC5B*  
epigenetic regulation. The DNMT1 effect was potentiated  
by HDAC2. In the same studies it was found that methylation  
of the two promoters dramatically impaired their activation  
by Sp1 transcription factor. Finally, one of the most important  
findings in this study was that *MUC2* and *MUC5B* epigenetic  
regulation, and particularly the methylation profile of their  
promoters, was directly dependent on the cell differentiation  
status, leading to the hypothesis that hypermethylation of  
*MUC2* and *MUC5B* may be a specific mechanism used by  
epithelial cancer cells to maintain a proliferating undiffer-  
entiated state or by normal cells to maintain pluripotency  
during development [44].

300

### 2.1.2 Membrane-bound mucins

#### 2.1.2.1 MUC4

305 The 5'-flanking region of *MUC4* is characterized by a high  
G+C content, up to 72% concentrated almost exclusively in the  
proximal promoter and the 5'-UTR, and the presence of two  
CpG islands of 135 and 146 bp, respectively, both located in  
the proximal promoter at -2370/-2236 and -738/-593 [48].

310 The influence of histone deacetylation on *MUC4* silencing  
was first assessed by the authors' laboratory in the pancreatic  
cancer cell line PANC-1 using cell treatment with TSA [49]  
and later shown in prostate cancer cells treated with sodium  
butyrate by Singh and co-workers [50]. This group also  
showed the influence of DNA methylation in regulating  
*MUC4* in these cells by using 5-aza.

315 The studies in epithelial cancer cells that express variable  
levels of *MUC4* showed that *MUC4* proximal and distal  
promoters, including both CpG islands, were heavily methy-  
lated whether or not the gene was expressed in the cells. On  
the contrary, *MUC4* 5'-UTR, and particularly cytosines at  
320 -81, -93, -102, -113 and -121, showed a methylation profile  
that was directly correlated to the level of *MUC4* expression  
in the cells [48]. Hypermethylation of *MUC4* 5'-UTR was  
associated with the establishment of a repressive histone  
code, including deacetylation of histone H3 and methylation  
of lysines 9 and 27 of histone H3 [48]. As four Sp1 binding  
sites had previously been identified and located, just  
upstream of and within the 5'-UTR [51], the influence of  
methylation of these Sp1 *cis*-elements on the regulation of  
325 *MUC4* by this transcription factor after *in vitro* methylation

of the promoter coupled to site-directed mutagenesis of the  
Sp1 sites was investigated further. It was found that methy-  
lation of Sp1 binding sites located in the proximal promoter  
and the 5'-UTR at -276/-271 and -166/-156 dramatically  
impaired *MUC4* activation by Sp1, whereas methylation of  
the adjacent CpG site upstream of the 5'-UTR had no  
effect. Finally, siRNA approach and ChIP assays allowed us  
to identify DNMT3A, DNMT3B and HDAC3 as potent  
regulators of *MUC4* endogenous expression that directly  
bind to its 5'-UTR [48].

330 From these results, a model was proposed of *MUC4*  
epigenetic regulation in epithelial cells [48]. In normal pancreatic  
cells that do not express *MUC4* [52], *MUC4* silencing would  
be mediated by DNA methylation and histone deacetyla-  
tion at the 5'-UTR, whereas in early steps of pancreatic  
carcinogenesis progressive demethylation would allow low  
expression of *MUC4*, and finally in adenocarcinomatous  
high-expressing cells, selective hypomethylation and permissive  
chromatin would allow binding of essential transcription  
factors such as Sp1 and full transcription of the *MUC4*  
gene. Recently, Yamada and co-workers showed that the  
methylation level of cytosines at -170 and -160 was associ-  
ated with *MUC4* gene expression in 10 cancer cell lines using  
the MassARRAY compact system, and therefore confirmed  
the critical contribution of promoter CpG methylation in  
*MUC4* silencing [53].

#### 2.1.2.2 MUC1

360 The *MUC1* gene is characterized by the presence of a CpG  
island in a large transcribed region that consists of 60-bp  
tandemly repeated units, each containing 1 SmaI restriction  
site. The first evaluation of the methylation status of various  
regions of the *MUC1* gene by Zrihan-Licht and co-workers  
using the methylation-sensitive enzymes SmaI and XmaI  
showed that, in peripheral blood leukocytes that do not  
express *MUC1*, the 60-bp repeat array was completely methy-  
lated, whereas the same sequences were entirely unmethy-  
lated in DNA from *MUC1*-overexpressing breast tumor  
tissue. Hypomethylation within the repeat array was also  
observed in other epithelial tissues that express *MUC1*  
at much lower levels than those seen in breast cancer tissue,  
demonstrating that hypomethylation of the tandem repeat  
array was an absolute requirement for *MUC1* gene expres-  
sion in epithelial tissues [54]. The influence of epigenetics  
in *MUC1* silencing was then confirmed in prostate cancer  
cells by using cell treatment with 5-aza and sodium  
butyrate [50]. The precise mechanisms of epigenetic regula-  
tion at *MUC1* promoter had not been deciphered until  
recently, when Yamada and co-workers found hypermethyla-  
tion of cytosines at -70, -55, -51, -47, -31, -21, -14, -4 and +20,  
tightly associated with dimethylation of H3-K9, in most  
*MUC1* non-expressing cell lines, providing the first proof  
that *MUC1* gene expression was regulated by DNA methylation  
of the promoter and associated histone H3 lysine 9 (H3-K9)  
modification [55].



385 **2.2 Moving towards using mucin methylation as a diagnostic tool**

390 Bisulfite-treated DNA sequencing of single clones is a gold standard and has been a very useful tool in our comprehensive studies to analyse precisely the methylation profile of mucin gene promoters and to determine the key CpG sites to use mucin gene methylation as a biomarker. However, applica-  
 395 tion of this method in routine procedures for molecular diagnosis is constrained by several issues, as it is complex, time-consuming, expensive, and requires a fair number of sequenced clones to be considered quantitative. Therefore, our laboratory is currently validating the pyrosequencing technology to analyse the methylation status of mucin promoters in human tissues and validate the use of mucin gene methylation as a diagnostic and/or prognostic tool in  
 400 epithelial cancers. Pyrosequencing is a fast, robust and flexible sequencing-by-synthesis-based method that offers a reproducible quantification of individual, consecutive CpG sites (up to 100 bp amplicon length), enabling discrimination of small changes in methylation levels, from single cytosines to  
 405 global methylation of a promoter region, in a large number of samples [56].

This switch to pyrosequencing seems very promising, as our first data for *MUC5B* (this review), *MUC5AC* and *MUC4* (unpublished data) already allowed us to confirm the potent status of CpG methylation as a biological tool in lung, colon and pancreatic cancer, respectively. The high level of methylation of *MUC5B* distal promoter seen in a *MUC5B* non-expressing normal lung tissue by bisulfite DNA sequencing is even more important in the correspond-  
 410 ing *MUC5B* non-expressing lung adenocarcinoma (Figure 2A). In another histologic type of lung cancer, a *MUC5B* non-expressing squamous cell lung carcinoma, it was also found, using pyrosequencing, that *MUC5B* distal promoter was hypermethylated at cytosines -2430, -2424, and -2421 when compared with normal lung tissue (Figure 2B). Regarding  
 415 *MUC4*, our first analysis of its pattern of methylation in normal and cancerous pancreatic tissues confirmed that demethylation of cytosines at -93, -102 and -113 occurs in pancreatic adenocarcinoma when compared with *MUC4*-non-expressing normal pancreas (not shown). Although  
 420 these preliminary data need further confirmation on larger panels of tissues, it is hoped to provide a rapid way of evaluating mucin gene methylation in cohorts and use it as a specific biomarker. As mucin genes show altered expres-  
 425 sion in various types of epithelial cancer (adenocarcinoma, squamous carcinoma), and also in numerous organs, tissues (respiratory tract, gastrointestinal tract, urogenital tract, etc.) and fluids (tears, saliva, sperm, milk, etc.), a lot of work as yet to be done. Moreover, in the future this method should  
 430 be of great help to determine the prevalence of mucin gene methylation in genomic DNA released from tumors in biological fluids such as plasma or serum, bronchoalveolar lavages for lung cancer, and pancreatic juice for pancreatic cancer.  
 435  
 439

## 2.3 Mucins and epigenomics

440 All the techniques that were used to study epigenetic changes at promoters of candidate genes can now be coupled both with genome-wide approaches, such as microarray technologies, and high-throughput methods, such as ultradeep sequencing, for high-resolution mapping of epigenetic profiles in both  
 445 normal and cancer cell types [57,58]. *Methods* for genome-wide mapping of DNA methylation patterns include methylated CpG island amplification (MCA), followed by hybridization on microarrays [59], methylated DNA immunoprecipitation (MeDIP)-on-chip [60], or ultradeep bisulfite sequencing [61].  
 450 Similarly, histone modification status can be widely studied using chromatin-immunoprecipitation-based methods [13,62] such as ChIP-on-chip or ChIP-seq. These new technologies will allow us to decipher further the mechanisms by which mucin genes are epigenetically regulated as clusters. Indeed,  
 455 regulation of the 11p15 MUC cluster at the gene level has been studied previously in the authors' laboratory, but has not yet been completely understood at a more global level. Similarly, regulation of mucin gene clusters at 3q29 (*MUC4*, *MUC20*) and 7q22 (*MUC3*, *MUC12*, *MUC17*) chromosomal  
 460 regions [26] remains to be investigated.

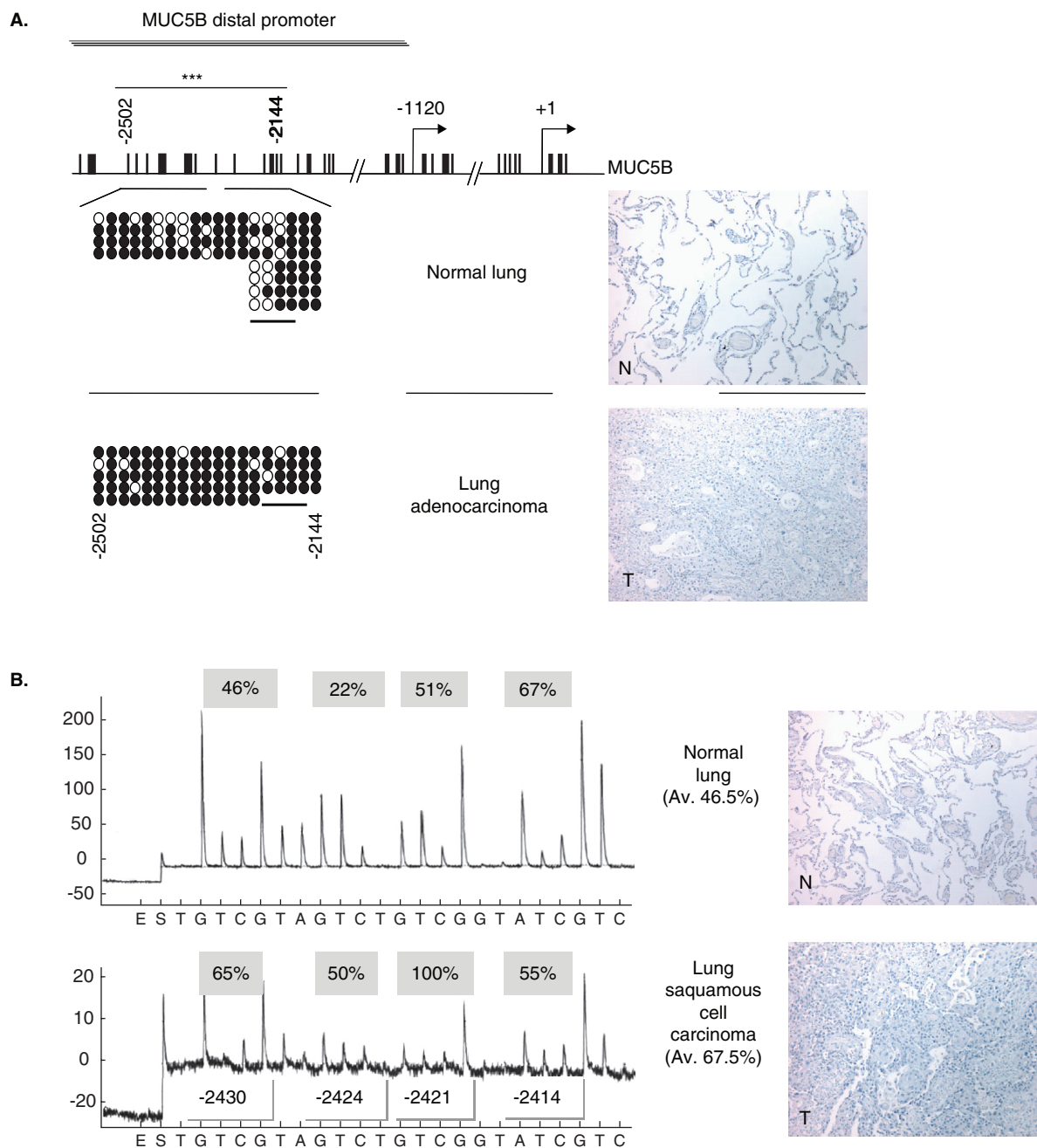
These genome-wide approaches will also be essential and necessary if one wants to give an explanation of the never-answered hypothesis as to how the tight coordination works and leads to simultaneous expression of a specific panel of  
 465 both secreted and membrane-bound mucins, which follow two different biosynthetic pathways, in each type of epithelium. Finally, these comprehensive studies will allow us to understand better the specific mechanisms responsible for main-  
 470 taining epithelium protection, as mucins are key proteins in epithelial homeostasis, and, therefore, the potential impacts of the dysregulation of their genes in epithelial diseases.

Most importantly, as it is now agreed that individual biomarkers are not informative enough for the diagnosis of most tumor types, translational epigenomics will be an  
 475 essential tool to determine an ideal set of various biomarkers (tumor ID or tumor signature), which will include specific epigenetic modifications of mucin genes, that may define each neoplasia and therefore be used for diagnosis and prognosis of epithelial cancers.  
 480

## 2.4 Mucins and regulating RNAs

## 2.4.1 MicroRNAs

485 MicroRNAs are endogenous non-coding RNAs, of 18 – 24 nucleotides in length, typically excised from 60 to 110 nucleotide foldback RNA precursor structures [63], that play important negative regulatory roles in animals and plants by targeting messenger RNA transcripts for degradation or translational repression, involving the RNA interference silencing complex (RISC) [10]. Recently, Gonzalez and co-workers  
 490 showed that miRNAs can also trigger transcriptional silencing by means of chromatin remodelling (i.e., trimethylation of H3K9) at DNA regions containing complementary sequences [64].  
 494



**Figure 2. Methylation profile of *MUC5B* distal promoter in *MUC5B* non-expressing lung cancers.** **A.** Mapping of methylated cytosines was carried out by bisulfite-treated DNA sequencing in normal lung (upper panel, N) and in a *MUC5B*-negative lung adenocarcinoma (lower panel, T). On the right-hand side is shown immunohistochemical staining of *MUC5B* apomucin in the corresponding tissues (magnification  $\times 100$ ). Partial sequence of *MUC5B* promoter is represented by the horizontal bar. Numbering refers to the proximal transcription start site of *MUC5B* (+1, broken arrow) [42,106]. Vertical bars indicate CpG sites. More than four individual clones were analysed per CpG site and type of tissue (normal lung or lung adenocarcinoma). Black circles represent methylated cytosines and open circles represent unmethylated cytosines. Key CpG methylated sites previously identified in cancer cell lines [44] are topped with a star and their position in the promoter is indicated. The horizontal thick bar indicates the promoter region analysed further using the pyrosequencing technology. **B.** Mapping of methylated cytosines in *MUC5B* promoter by pyrosequencing in normal lung (upper panel, N) and in a *MUC5B*-negative lung squamous cell carcinoma (lower panel, T). On the right-hand side is shown immunohistochemical staining of *MUC5B* apomucin in the corresponding tissues (magnification  $\times 100$ ). The pyrograms show the methylation percentages of the four CpG sites of interest (-2430, -2424, -2421 and -2414) in *MUC5B* distal promoter using the pyrosequencing technology (PyroMark™ MD, Biotage).

Av: The average methylation content of the promoter in normal lung compared with lung squamous cell carcinoma.

495 So far, hundreds of unique, mature miRNAs have been  
 identified in humans, with more to be discovered. Their expres-  
 sion levels vary greatly among tissues [65] and especially during  
 pathological processes [66], including carcinogenesis [12,67,68].  
 500 Chromosomal rearrangements, genomic amplifications or  
 deletions, epigenetic regulation and mutations at genes  
 encoding miRNAs lead to their specific overexpression or  
 underexpression in different types of tumor, which contributes  
 to cancer development and progression. Changes in miRNA  
 expression have been reported by various genome-wide techni-  
 505 ques (microarray platforms, bead-based flow cytometry) in col-  
 orectal neoplasia [69], pancreatic cancer [70-72], kidney and  
 bladder cancers [73], esophageal [74] and gastric carcinomas [75]  
 and lung cancer [76], potentially affecting expression levels of  
 numerous putative targets (Table 1). This led to the new concept  
 510 of miRNA expression signatures [75] associated with cancer  
 diagnosis (tissue type), predisposition, prognosis (differentiation  
 and aggressiveness, [77]), identification of a primary tumor [78]  
 and response to therapy [79]. Moreover, miRNAs are present  
 in human plasma in a remarkably stable form and therefore  
 515 represent a potentially useful approach for the biological  
 fluid-based detection of human cancer [80]. For most miRNAs  
 though, identification of their target genes and the biological  
 consequences of their repressive activity on cancer progression  
 remain to be defined.

520 No evidence for mucin gene regulation by miRNAs has  
 been reported so far. However, interrogation of MiRbase and  
 MiRanda databases ([81-84]) revealed a large number of  
 miRNAs that could potentially target 33-UTR of mucin  
 genes encoding either the membrane-bound mucins MUC1,  
 525 MUC4, MUC20, MUC3A/3B, MUC17 and MUC13 or  
 the secreted mucins MUC2, MUC5AC, MUC6, MUC7  
 and MUC19 (Table 2). Interestingly, although miRNAs  
 might be responsible for mucin gene regulation, a recent  
 study by Ramasamy and co-workers showed, conversely, that  
 530 *N*-glycosylation of Asn-36 on MUC1 C-terminal subunit  
 suppresses miR-322 expression in breast and prostate cancer  
 cells, thereby increasing expression of galectin-3 [85]. This  
 very interesting result suggests that, in turn, mucins may  
 regulate miRNA expression and activity.

535 **2.4.2 Antisense RNA**

Natural antisense transcripts are endogenous RNA molecules  
 containing sequences that are complementary to the protein-  
 encoding sense transcript. *Cis* antisense RNAs are transcribed  
 540 at the same genomic locus from the opposite strand, whereas  
*trans* antisense RNA are transcribed from separate loci.  
 Antisense RNAs are thought to regulate their target genes at  
 the genomic level by triggering chromatin remodelling [86], or at  
 the transcriptomic level by interfering with mRNA transcription,  
 545 processing, stability, translocation and translation [87].

Recently, evidence for antisense transcripts has been  
 reported in 2900 – 6400 human genes using the asymmetric  
 strand-specific analysis of gene expression (ASSAGE)  
 549 technology [88]. The distribution of these antisense RNAs

is non-random across the genome and differs among cell types, 550  
 suggesting a fundamental role in regulation of sense tran-  
 scription. In this comprehensive study, sense and antisense  
 expression of 32,354 Ensembl genes in three cancerous and  
 two normal cell lines was assessed, including those of *MUC1*,  
 555 *MUC4*, *MUC3A/3B*, *MUC17*, *MUC16*, *MUC2*, *MUC5AC*,  
*MUC5B*, *MUC6* and *MUC7*. The results show that high  
 expression of *MUC5B* and *MUC6* antisense RNAs (Figure 1)  
 was found in non-epithelial cells that do not express sense  
 transcripts of these genes. These preliminary data concerning  
 expression of mucin antisense RNAs will need further inves-  
 560 tigation. Moreover, as epigenetic silencing of tumor suppressor  
 genes by their antisense RNA has already been reported, the  
 role of these transcripts in mucin gene silencing by methylation  
 and histone modifications will have to be elucidated.

565 **3. Expert opinion**

It is obvious that epigenetic regulation of mucin genes is  
 still at its dawn, but *in vitro* studies in cancer cell lines,  
 expression studies on human tissue sections and very recent 570  
 genome-wide studies using new technologies clearly indicate  
 that DNA methylation of their promoters is a key molecular  
 mechanism regulating their expression in epithelial cancers.  
 Detection of MUC methylation may thus soon provide a  
 useful biological tool for the clinician. The greatest challenge 575  
 for researchers working in the mucin field is of course  
 related to the huge diversity of these glycoproteins and also  
 their genes (> 20 known until now), the huge size of the  
 genes, their polymorphisms, and the redundancy that such a  
 complex family of molecules may create. 580

We think that systematic comparison of MUC methylation  
 in normal and carcinomatous tissues will provide useful  
 tools for diagnosis and prognosis of numerous epithelial  
 cancers, including those of lung [89], pancreas [90] and  
 colon [91], but also esophageal and gastric carcinoma. Altera- 585  
 tions in DNA methylation are early events in carcinogenesis  
 and, as such, could become specific and sensitive bio-  
 markers that could be detected in biological fluids such as  
 blood (liver cancer), sputum and bronchoalveolar lavage  
 (lung cancer), feces (colon cancer), urine (bladder cancer) or 590  
 nipple aspirate (breast cancer) [5,92]. However, so far, methy-  
 lation status for one particular gene as a reliable marker has  
 been described only in rare cases (*GSTP1* in prostate cancer [93]  
 or *APC* in esophageal adenocarcinoma [94]). Most of the  
 time, analysis of several genes (methylome signature) is 595  
 necessary to distinguish cancers from normal tissues or  
 other pathologies [88,95]. Moreover, as methylation is a  
 tumor-specific change in DNA, identification of panels of  
 complementary biomarkers may help to classify histopatho-  
 logic tumor types, tumor grades as well as tumors from 600  
 different tissues [13]. Thus, methylation of genes encoding  
 mucins, which are markers of cell differentiation in many  
 epithelial tissues, may provide a very useful tool to clinicians  
 and pathologists. 604

**Table 1. Putative target genes of differentially expressed miRNAs identified in aerodigestive cancers.**

| miRNA        | Chromosomal localization | Putative targets  | Tumor type  |
|--------------|--------------------------|---|---|
| let-7 family | Multiple loci            | Ras <sup>‡</sup> , c-myc <sup>‡</sup> , HMGA2, IMP-1, ARID3B, HIC2, GM632 | Kidney, colon, lung <sup>§</sup>                        |
| miR-100      | 11q24.1                  | FZD8, INSM1, SMARCA5, ZZEF1, FRAP1, TRIB2, HOXA1, FGF3                    | Esophagus   |
| miR-103-1    | 5q35.1                   | RAB10, ZHX1, OGT, HSH1A, BACH2  | Bladder   |
| miR-106a     | Xq26.2                   | MAP3K9, MAP3K11, MAPK9, FASTK, ARHGAP12, BCL2L11                          | Colon, pancreas   |
| miR-107      | 10q23.31                 | FGFR2, CCNE1, MAP2K3  | Colon, pancreas, stomach                                |
| miR-125b     | 11q24.1                  | BAK1, MIZF, MAP2K7, RAB6B, MMP11, MADD                                    | Esophagus   |
| miR-143      | 5q32-33                  | MAPK7 <sup>‡</sup> , MAP3K7, KRAS, HOXA5                                  | Colon   |
| miR-146a     | 5q33.3                   | IRAK1   | Pancreas  |
| miR-152      | 17q21.32                 | ELF5, TGIF2, DNMT1, CDC2L5  | Esophagus   |
| miR-155      | 21q21.3                  | PLR3 <sup>‡</sup> , FGF7, GDF6, TP53INP1                                  | Colon, lung <sup>§</sup> , stomach                      |
| miR-17-5p    | 13q31                    | ZBTB4, ITGB8, EZH1, KIAA1404, GM632                                       | Bladder, colon, lung, pancreas                          |
| miR-181b-1   | 1q31.3                   | ADARB1, CTDSPL, PAWR, PHF15, TNFSF11                                      | Pancreas  |
| miR-185      | 22q11.2                  | ZNF1A4, SLC16A2, PCDHAC2, PCDHAC1, PCDHA8                                 | Kidney, bladder   |
| miR-191      | 3p21.31                  | GAP43, EGR1, MAP3K12, NRCAM, CCND2, FZD5, PLCD1                           | Colon, lung, pancreas, stomach                          |
| miR-192      | 11q13.1                  | ATF1, CCNT2, RAB2   | Esophagus   |
| miR-194      | 1q41                     | CROP, TGIF1   | Esophagus   |
| miR-200c     | 12p13.31                 | RELN, PDCD10, PCDH8   | Esophagus   |
| miR-203      | 14q32.33                 | ZNF281, RKHD2, MGC39518, TRPS1, LPIN1                                     | Bladder, esophagus                                      |
| miR-205      | 1q32.2                   | PLCB1, CPEB2, MGRN1, ESRRG, CHN1  | Bladder, esophagus                                      |
| miR-21       | 17q23.1                  | BCL2, NTF3, PDCD4, RAB6A, RAB6C, RASA1, RHOB, SKI, TGFB1, TGFB2, TIMP3    | Colon, lung, pancreas <sup>§</sup> , stomach, esophagus |
| miR-214      | 1q24.3                   | FGFR2, IGF1R, RAB14, RAB15  | Pancreas, stomach                                       |
| miR-221      | Xp11.3                   | RIMS3, HECTD2, CDKN1B, RBM24, ZFPM2                                       | Bladder, colon, pancreas, stomach                       |
| miR-223      | Xq12-13.3                | MEF2C, ZCCHC14, UE2A, SPRED1, SACS  | Bladder, colon, pancreas, stomach                       |
| miR-23a      | 19p13.2                  | CCNT2, TOP1, SATB1, SEMA6D, NEK6  | Bladder   |
| miR-23b      | 9q22.31                  | CCNT2, TOP1, SATB1, SEMA6D, NEK6  | Bladder   |

<sup>‡</sup>Identified targets.

<sup>§</sup>Prognostic and/or diagnostic markers.

Adapted from Gottardo *et al.* [73], Volinia *et al.* [75], Feber *et al.* [74] and Calin and Croce [63].

Target prediction is based on miRBase, TargetScan and MiRanda. The listed miRNAs are predicted by at least two of these prediction programs. The most likely tumor-related genes are listed.

**Table 1. Putative target genes of differentially expressed miRNAs identified in aerodigestive cancers (continued).**

| MiRNA     | Chromosomal localization | Putative targets   | Tumor type               |
|-----------|--------------------------|--|--------------------------|
| miR-24-1  | 9q22.32                  | BCL2L11, TRAF7, CREBL2, IGFBP5, PCDH10, TP53INP1, RASA1, MAPK7                     | Colon, pancreas, stomach |
| miR-25    | 7q22.1                   | PCDH11X, RAB23, FZD10, CDH10, E2F3   | Pancreas, stomach        |
| miR-26b   | 2q35                     | NHS, HSHIN1, LARP1, LOC153222, DDX3X   | Bladder                  |
| miR-28    | 3q28                     | SSRP1, SFRS5, RHO, GPM6A, EN2  | Kidney                   |
| miR-29b-2 | 1q32.2                   | COL3A1, COL4A1, COL4A5, COL7A1, COL2A1, COL15A1, TFAP2C, CCNYL1, NKRF              | colon, pancreas          |
| miR-30c   | 1p34.2                   | PCDH10   | Colon, pancreas          |
| miR-32    | 9q31.3                   | RAB14, RAB23, RAP1B, CREM, MYCBP2, FZD10, CREB1, SMAD7, NOTCH1, PAX3, SMAD6, GAP43 | Colon, pancreas          |
| miR-7-2   | 15q25                    | SPATA2, OGT, POLE4, RSBN1, KIAA1920  | Kidney                   |
| miR-93    | 7q22.1                   | CREBL1, MYCN, MAPK9  | Esophagus                |

\*Identified targets.

§Prognostic and/or diagnostic markers.

Adapted from Gottardo *et al.* [73], Volinia *et al.* [75], Feber *et al.* [74] and Calin and Croce [63].

Target prediction is based on miRBase, TargetScan and MiRanda. The listed miRNAs are predicted by at least two of these prediction programs The most likely tumor-related genes are listed.

605 Yet, this potential future promising clinical application  
 depends crucially on the discovery of useful aberrantly  
 methylated sequences. In this context, studying the methyl-  
 ation status of mucin genes, which are aberrantly expressed in  
 cancers, may help to develop these early detection tools. The  
 610 development of new powerful, reliable and rapid methods,  
 such as pyrosequencing or high-throughput technologies on  
 chips [13,96], to screen methylated genes in tissues should  
 quickly provide new information regarding mucin gene epi-  
 genetic status in tissues from cancer patients and at different  
 615 stages of carcinogenesis.

The studies of epigenetic regulation of mucin genes also  
 point out the opposite role of secreted and membrane-bound  
 mucins in carcinogenesis. Indeed, genes encoding secreted  
 mucins, and especially MUC2 and MUC5B, are often  
 620 aberrantly silenced in non-mucinous colon [97] and lung [98]  
 carcinomas, respectively, whereas genes encoding MUC1  
 and MUC4 membrane-bound mucins are overexpressed  
 in cancers particularly in breast [99] and pancreatic [100]  
 carcinomas, respectively. Moreover, in cancers, expression of  
 625 secreted mucins is often associated with better prognosis [101],  
 whereas expression of membrane-bound mucins is associ-  
 ated with tumor cell detachment, invasion and metastasis  
 and worse prognosis [99,102]. These data are in accordance  
 with the findings that MUC2 and MUC5B promoters  
 630 are hypermethylated, whereas those of MUC1 and MUC4  
 631 are demethylated in cancers. Aberrant silencing of specific

genes by hypermethylation and ectopic onco-fetal expres- 632  
 sion of other genes by demethylation are common features  
 of cancer cells and lead to selective advantage in term of  
 growth and survival. Thus, epigenetics provide a new 635  
 generation of tumor suppressor genes and oncogenes [103].  
 Therefore, studying epigenetic regulation of mucin genes  
 may help clarify their role in carcinogenesis as candidate  
 tumor suppressor genes, as has already been suggested for 640  
 Muc2 [35], or oncogenes, as has already been proposed for  
 MUC1 [104].

Finally, the findings that mucin genes may be epigeneti-  
 cally regulated in cancers prompt us to consider them as  
 attractive targets for new therapeutic strategies. As epigenetic  
 changes are potentially reversible, an increasing number of 645  
 specific DNA methylation or histone deacetylation pharma-  
 cological inhibitors have been developed over the past few  
 years. Among them, the DNMT inhibitor 5-aza (decitabine)  
 is the most extensively studied and has been shown to reac-  
 tivate MUC2 and MUC5B *in vitro* in numerous cancer cell 650  
 lines [44]. On the other hand, the HDAC inhibitor TSA,  
 which is known to induce apoptosis and enhance the  
 response to chemotherapeutic agents in pancreatic cancer [105],  
 has been shown to induce a severe decrease of MUC4  
 expression in pancreatic cancer cells [48]. Hence, in the future, 655  
 potential application of epigenetic drugs in either reactivating  
 expression of secreted mucins or inhibiting membrane-bound  
 mucins will have to be investigated. 658



**Table 2. Predicted miRNA targeting mucin genes.**

| Mucin gene                   | Chromosomal localization | Number of predicted miRNA | List of iRNA   |
|------------------------------|--------------------------|---------------------------|--|
| <b>Membrane-bound mucins</b> |                          |                           |  |
| <i>MUC1</i>                  | 1q21                     | 27                        | miR-183<br>miR-193b<br>miR-219-2-3p<br>miR-296-5p<br>miR-320<br>miR-328<br>miR-331-5p<br>miR-367<br>miR-371-5p<br>miR-485-5p<br>miR-502-5p<br>miR-509-3p<br>miR-512-5p<br>miR-519e<br>miR-548b-3p<br>miR-562<br>miR-587<br>miR-588<br>miR-612<br>miR-619<br>miR-647<br>miR-652<br>miR-668<br>miR-760<br>miR-885-5p<br>miR-892b<br>miR-923  |
| <i>MUC4</i>                  | 3q29                     | 52 <sup>†</sup>           | let-7 family*<br>miR-10a<br>miR-122<br>miR-138<br>miR-139-3p<br>miR-145<br>miR-147, miR-147b<br>miR-16-1<br>miR-193a-3p<br>miR-197<br>miR-199a-5p,<br>miR-199b-5p<br>miR-204<br>miR-20a*<br>miR-210<br>miR-211<br>miR-212<br>miR-219-1-3p<br>miR-297<br>miR-324-5p<br>miR-335<br>miR-373<br>miR-384<br>miR-411<br>miR-491-5p<br>miR-500<br>miR-502-3p<br>miR-548b-5p,<br>miR-548c-5p |

<sup>†</sup>Predicted only by miRBase.

Target prediction is based on miRBase and miRanda. The listed miRNAs are predicted by the two prediction programs.



**Table 2. Predicted miRNA targeting mucin genes (continued).**

| Mucin gene     | Chromosomal localization | Number of predicted miRNA | List of iRNA  |
|----------------|--------------------------|---------------------------|---|
|                |                          |                           | miR-560<br>miR-566<br>miR-574-5p<br>miR-595<br>miR-611<br>miR-627<br>miR-632<br>miR-648<br>miR-652<br>miR-924<br>miR-933<br>miR-935<br>miR-98   |
| <i>MUC20</i>   | 3q29                     | 15                        | miR-125a-3p<br>miR-129-3p<br>miR-205*<br>miR-222<br>miR-326<br>miR-330-5p<br>miR-455-3p,<br>miR-455-5p<br>miR-486-3p<br>miR-552<br>miR-588<br>miR-603<br>miR-611<br>miR-622<br>miR-647          |
| <i>MUC3A/B</i> | 7q22                     | 13                        | let-7 family*<br>miR-154<br>miR-196a,<br>miR-196b<br>miR-487a<br>miR-98   |
| <i>MUC17</i>   | 7q22                     | 7                         | miR-302b,<br>miR-302c,<br>miR-302d<br>miR-450b-3p<br>miR-486-5p<br>miR-769-3p<br>miR-95   |
| <i>MUC13</i>   | 3q13                     | 16                        | miR-132<br>miR-133a,<br>miR-133b<br>miR-181a,<br>miR-181b*,<br>miR-181c ,<br>miR-181d<br>miR-211<br>miR-30a, miR-30d<br>miR-335<br>miR-485-5p<br>miR-520d-3p,<br>miR-520e<br>miR-543<br>miR-633 |

\*Predicted only by miRBase.

Target prediction is based on miRBase and miRanda. The listed miRNAs are predicted by the two prediction programs.

Table 2. Predicted miRNA targeting mucin genes (continued).

| Mucin gene               | Chromosomal localization | Number of predicted miRNA | List of iRNA  |
|--------------------------|--------------------------|---------------------------|---|
|                          |                          |                           | miR-485-5p<br>miR-520d-3p,<br>miR-520e<br>miR-543<br>miR-633  |
| <b>Secreted mucins</b>   |                          |                           |   |
| <b>11p15 mucin genes</b> |                          |                           |   |
| <i>MUC2</i>              | 11p15                    | 15 <sup>‡</sup>           | miR-128a<br>miR-25*<br>miR-27a<br>miR-296-3p<br>miR-32*<br>miR-380<br>miR-431<br>miR-448<br>miR-453<br>miR-455-5p<br>miR-512-5p<br>miR-557<br>miR-646<br>miR-675<br>miR-92b   |
| <i>MUC5AC</i>            | 11p15                    | 10                        | miR-193a-3p,<br>miR-193b<br>miR-214*<br>miR-423-3p<br>miR-612<br>miR-638<br>miR-647<br>miR-663<br>miR-892b<br>miR-922   |
| <i>MUC5B</i>             | 11p15                    | 0                         | -   |
| <i>MUC6</i>              | 11p15                    | 23 <sup>‡</sup>           | miR-10a<br>miR-139-3p<br>miR-146b-3p<br>miR-184<br>miR-206<br>miR-298<br>miR-323-5p<br>miR-342-5p<br>miR-34a<br>miR-370<br>miR-432<br>miR-483-5p<br>miR-485-5p<br>miR-486-5p<br>miR-492<br>miR-574-5p<br>miR-585<br>miR-592<br>miR-595<br>miR-616 |

<sup>‡</sup>Predicted only by miRBase.

Target prediction is based on miRBase and miRanda. The listed miRNAs are predicted by the two prediction programs.

**Table 2. Predicted miRNA targeting mucin genes (continued).**

| Mucin gene                   | Chromosomal localization | Number of predicted miRNA | List of iRNA                                       |
|------------------------------|--------------------------|---------------------------|--|
|                              |                          |                           | miR-765<br>miR-890<br>miR-936                      |
| <b>Other secreted mucins</b> |                          |                           |  |
| <i>MUC7</i>                  | 4q13-q21                 | 2                         | miR-199a-3p<br>miR-544                             |
| <i>MUC19</i>                 | 12q12                    | 9                         | miR-140-5p<br>miR-146a*,<br>miR-146b-5p<br>miR-29b |

\*Predicted only by miRBase.  
Target prediction is based on miRBase and miRanda. The listed miRNAs are predicted by the two prediction programs.

659 Deciphering the epigenetic mechanisms regulating mucin  
660 gene expression has been very helpful in understanding  
mucin gene biology better, but in the future it will provide  
clinicians and pathologists with new biological tools to  
screen patients and detect cancer at an early stage as well as  
classify tumors and propose new therapeutic strategies.

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**Declaration of interest**

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**Bibliography**

- Russo VEA, Martienssen RA, Riggs AD. Epigenetic mechanisms of gene regulation. New York: Cold Spring Harbor Laboratory Press, 1996
- Tost J. Epigenetics. Norfolk: Caister Academic Press, 2008
- Robertson KD. DNA methylation and human disease. Nat Rev Genet 2005;6(8):597-610
- Chen T, Ueda Y, Dodge JE, et al. Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. Mol Cell Biol 2003;23(16):5594-605
- Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. Biochim Biophys Acta 2007;1775(1):138-62
- Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349(21):2042-54
- Razin A. CpG methylation, chromatin structure and gene silencing—a three-way connection. Embo J 1998;17(17):4905-8
- Cervoni N, Szyf M. Demethylase activity is directed by histone acetylation. J Biol Chem 2001;276(44):40778-87
- Mutskov V, Felsenfeld G. Silencing of transgene transcription precedes methylation of promoter DNA and histone H3 lysine 9. Embo J 2004;23(1):138-49
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116(2):281-97
- He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004;5(7):522-31
- Wiemer EA. The role of microRNAs in cancer: no small matter. Eur J Cancer 2007;43(10):1529-44
- Shames DS, Minna JD, Gazdar AF. Methods for detecting DNA methylation in tumors: from bench to bedside. Cancer Lett 2007;251(2):187-98
- Teodoridis JM, Strathdee G, Brown R. Epigenetic silencing mediated by CpG island methylation: potential as a therapeutic target and as a biomarker. Drug Resist Updat 2004;7(4-5):267-78
- Palmisano WA, Divine KK, Saccomanno G, et al. Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Res 2000;60(21):5954-8
- Worm J, Guldberg P. DNA methylation: an epigenetic pathway to cancer and a promising target for anticancer therapy. J Oral Pathol Med 2002;31(8):443-9
- Strathdee G, Brown R. Epigenetic cancer therapies: DNA methyltransferase inhibitors. Expert Opin Investig Drugs 2002;11(6):747-54
- Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. Nat Rev Drug Discov 2006;5(1):37-50
- Liang JJ, Kimchi ET, Staveley-O'Carroll KF, Tan D. Diagnostic and prognostic biomarkers in pancreatic carcinoma. Int J Clin Exp Pathol 2009;2(1):1-10

20. Van Seuningen I, Pigny P, Perrais M, et al. Transcriptional regulation of the 11p15 mucin genes. Towards new biological tools in human therapy, in inflammatory diseases and cancer? *Front Biosci* 2001;6:D1216-34
21. Mall AS. Analysis of mucins: role in laboratory diagnosis. *J Clin Pathol* 2008;61(9):1018-24
22. Leteurtre E, Piessen G, Aubert S, et al. Mucins as diagnostic and/or prognostic factors in epithelial cancers. In: Van Seuningen I, editor, *The epithelial mucins: structure/function Roles in cancer and inflammatory diseases*. Kerala: Research Signpost; 2008. p. 273-94
23. Pigny P, Guyonnet-Duperat V, Hill AS, et al. Human mucin genes assigned to 11p15.5: identification and organization of a cluster of genes. *Genomics* 1996;38(3):340-52
24. de Bustros A, Nelkin BD, Silverman A, et al. The short arm of chromosome 11 is a 'hot spot' for hypermethylation in human neoplasia. *Proc Natl Acad Sci USA* 1988;85(15):5693-7
25. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004;4(1):45-60
26. Jonckheere N, Van Seuningen I. The ever growing family of membrane-bound mucins. In: Van Seuningen I, editor, *The epithelial mucins: structure/function Roles in cancer and inflammatory diseases*. Kerala: Research Signpost; 2008. p. 17-38
27. Yonezawa S, Goto M, Yamada N, et al. Expression profiles of MUC1, MUC2, and MUC4 mucins in human neoplasms and their relationship with biological behavior. *Proteomics* 2008;8(16):3329-41
28. Gicquel C, Rossignol S, Cabrol S, et al. Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. *Nat Genet* 2005;37(9):1003-7
29. Schonherr N, Meyer E, Roos A, et al. The centromeric 11p15 imprinting centre is also involved in Silver-Russell syndrome. *J Med Genet* 2007;44(1):59-63
30. Weksberg R, Smith AC, Squire J, Sadowski P. Beckwith-Wiedemann syndrome demonstrates a role for epigenetic control of normal development. *Hum Mol Genet* 2003;12 Spec No 1:R61-8
31. Craig JM, Bickmore WA. The distribution of CpG islands in mammalian chromosomes. *Nat Genet* 1994;7(3):376-82
32. Nakano S, Murakami K, Meguro M, et al. Expression profile of LIT1/KCNQ1OT1 and epigenetic status at the KvDMR1 in colorectal cancers. *Cancer Sci* 2006;97(11):1147-54
33. Sato N, Matsubayashi H, Abe T, et al. Epigenetic down-regulation of CDKN1C/p57KIP2 in pancreatic ductal neoplasms identified by gene expression profiling. *Clin Cancer Res* 2005;11(13):4681-8
34. Schwienbacher C, Gramantieri L, Scelfo R, et al. Gain of imprinting at chromosome 11p15: a pathogenetic mechanism identified in human hepatocarcinomas. *Proc Natl Acad Sci USA* 2000;97(10):5445-9
35. Velcich A, Yang W, Heyer J, et al. Colorectal cancer in mice genetically deficient in the mucin *Muc2*. *Science* 2002;295(5560):1726-9
36. Hanski C, Riede E, Gratchev A, et al. MUC2 gene suppression in human colorectal carcinomas and their metastases: in vitro evidence of the modulatory role of DNA methylation. *Lab Invest* 1997;77(6):685-95
37. Gratchev A, Siedow A, Bumke-Vogt C, et al. Regulation of the intestinal mucin MUC2 gene expression in vivo: evidence for the role of promoter methylation. *Cancer Lett* 2001;168(1):71-80
38. Siedow A, Szyf M, Gratchev A, et al. De novo expression of the *Muc2* gene in pancreas carcinoma cells is triggered by promoter demethylation. *Tumour Biol* 2002;23(1):54-60
39. Mesquita P, Peixoto AJ, Seruca R, et al. Role of site-specific promoter hypomethylation in aberrant MUC2 mucin expression in mucinous gastric carcinomas. *Cancer Lett* 2003b;189(2):129-36
40. Hamada T, Goto M, Tsutsumida H, et al. Mapping of the methylation pattern of the MUC2 promoter in pancreatic cancer cell lines, using bisulfite genomic sequencing. *Cancer Lett* 2005;227(2):175-84
41. Yamada N, Hamada T, Goto M, et al. MUC2 expression is regulated by histone H3 modification and DNA methylation in pancreatic cancer. *Int J Cancer* 2006;119(8):1850-7
42. Perrais M, Pigny P, Buisine MP, et al. Aberrant expression of human mucin gene MUC5B in gastric carcinoma and cancer cells. Identification and regulation of a distal promoter. *J Biol Chem* 2001;276(18):15386-96
43. Ho JJ, Han SW, Pan PL, et al. Methylation status of promoters and expression of MUC2 and MUC5AC mucins in pancreatic cancer cells. *Int J Oncol* 2003;22(2):273-9
44. Vincent A, Perrais M, Desseyn JL, et al. Epigenetic regulation (DNA methylation, histone modifications) of the 11p15 mucin genes (MUC2, MUC5AC, MUC5B, MUC6) in epithelial cancer cells. *Oncogene* 2007;26(45):6566-76
45. Augenlicht L, Shi L, Mariadason J, et al. Repression of MUC2 gene expression by butyrate, a physiological regulator of intestinal cell maturation. *Oncogene* 2003;22(32):4983-92
46. Mayo C, Lloreta J, Real FX, Mayol X. In vitro differentiation of HT-29 M6 mucus-secreting colon cancer cells involves a trichostatin A and p27(KIP1)-inducible transcriptional program of gene expression. *J Cell Physiol* 2007;212(1):42-50
47. Smiraglia DJ, Rush LJ, Fruhwald MC, et al. Excessive CpG island hypermethylation in cancer cell lines versus primary human malignancies. *Hum Mol Genet* 2001;10(13):1413-9
48. Vincent A, Ducourouble MP, Van Seuningen I. Epigenetic regulation of the human mucin gene MUC4 in epithelial cancer cell lines involves both DNA methylation and histone modifications mediated by DNA methyltransferases and histone deacetylases. *FASEB J* 2008;22(8):3035-45
49. Jonckheere N, Perrais M, Mariette C, et al. A role for human MUC4 mucin gene, the ErbB2 ligand, as a target of TGF-beta in pancreatic carcinogenesis. *Oncogene* 2004;23(34):5729-38
50. Singh AP, Chauhan SC, Bafna S, et al. Aberrant expression of transmembrane mucins, MUC1 and MUC4, in human prostate carcinomas. *Prostate* 2006;66(4):421-9
51. Perrais M, Pigny P, Ducourouble MP, et al. Characterization of human mucin gene MUC4 promoter: importance of growth factors and proinflammatory cytokines for its regulation in pancreatic cancer cells. *J Biol Chem* 2001;276(33):30923-33
52. Fauquette V, Aubert S, Groux-Degroote S, et al. Transcription factor AP-2alpha represses both the mucin MUC4 expression

## Mucins: a new family of epigenetic biomarkers in epithelial cancers

- and pancreatic cancer cell proliferation. *Carcinogenesis* 2007;28(11):2305-12
53. Yamada N, Nishida Y, Tsutsumida H, et al. Promoter CpG methylation in cancer cells contributes to the regulation of MUC4. *Br J Cancer* 2009
  54. Zrihan-Licht S, Weiss M, Keydar I, Wreschner DH. DNA methylation status of the MUC1 gene coding for a breast-cancer-associated protein. *Int J Cancer* 1995;62(3):245-51
  55. Yamada N, Nishida Y, Tsutsumida H, et al. MUC1 expression is regulated by DNA methylation and histone H3 lysine 9 modification in cancer cells. *Cancer Res* 2008;68(8):2708-16
  56. Tost J, Gut IG. DNA methylation analysis by pyrosequencing. *Nat Protoc* 2007;2(9):2265-75
  57. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007;8(4):286-98
  58. Zilberman D, Henikoff S. Genome-wide analysis of DNA methylation patterns. *Development* 2007;134(22):3959-65
  59. Omura N, Li CP, Li A, et al. Genome-wide profiling of methylated promoters in pancreatic adenocarcinoma. *Cancer Biol Ther* 2008;7(7):1146-56
  60. Keshet I, Schlesinger Y, Farkash S, et al. Evidence for an instructive mechanism of de novo methylation in cancer cells. *Nat Genet* 2006;38(2):149-53
  61. Taylor KH, Kramer RS, Davis JW, et al. Ultradeep bisulfite sequencing analysis of DNA methylation patterns in multiple gene promoters by 454 sequencing. *Cancer Res* 2007;67(18):8511-8
  62. Massie CE, Mills IG. ChIPping away at gene regulation. *EMBO Rep* 2008;9(4):337-43
  63. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006;6(11):857-66
  64. Gonzalez S, Pisano DG, Serrano M. Mechanistic principles of chromatin remodeling guided by siRNAs and miRNAs. *Cell Cycle* 2008;7(16):2601-8
  65. Wang H, Ach RA, Curry B. Direct and sensitive miRNA profiling from low-input total RNA. *RNA* 2007;13(1):151-9
  66. Wang Y, Liang Y, Lu Q. MicroRNA epigenetic alterations: predicting biomarkers and therapeutic targets in human diseases. *Clin Genet* 2008;74(4):307-15
  67. Lee YS, Dutta A. MicroRNAs in Cancer. *Annu Rev Pathol* 2008
  68. Zhang B, Farwell MA. microRNAs: a new emerging class of players for disease diagnostics and gene therapy. *J Cell Mol Med* 2008;12(1):3-21
  69. Asangani IA, Rasheed SA, Nikolova DA, et al. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene* 2008;27(15):2128-36
  70. Bloomston M, Frankel WL, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 2007;297(17):1901-8
  71. Lee EJ, Gusev Y, Jiang J, et al. Expression profiling identifies microRNA signature in pancreatic cancer. *Int J Cancer* 2007;120(5):1046-54
  72. Szafranska AE, Davison TS, John J, et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene* 2007;26(30):4442-52
  73. Gottardo F, Liu CG, Ferracin M, et al. Micro-RNA profiling in kidney and bladder cancers. *Urol Oncol* 2007;25(5):387-92
  74. Feber A, Xi L, Luketich JD, et al. MicroRNA expression profiles of esophageal cancer. *J Thorac Cardiovasc Surg* 2008;135(2):255-60; discussion 60
  75. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006;103(7):2257-61
  76. Mascaux C, Laes JF, Anthoine G, et al. Evolution of microRNAs expression during human bronchial squamous carcinogenesis. *Eur Respir J* 2008
  77. Lujambio A, Calin GA, Villanueva A, et al. A microRNA DNA methylation signature for human cancer metastasis. *Proc Natl Acad Sci USA* 2008;105(36):13556-61
  78. Rosenfeld N, Aharonov R, Meiri E, et al. MicroRNAs accurately identify cancer tissue origin. *Nat Biotechnol* 2008;26(4):462-9
  79. Love TM, Moffett HF, Novina CD. Not miR-ly small RNAs: big potential for microRNAs in therapy. *J Allergy Clin Immunol* 2008;121(2):309-19
  80. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008;105(30):10513-8
  81. Available from: <http://microrna.sanger.ac.uk/targets>
  82. Available from: <http://www.microrna.org>
  83. Griffiths-Jones S, Grocock RJ, van Dongen S, et al. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 2006;34(Database issue):D140-4
  84. John B, Enright AJ, Aravin A, et al. Human MicroRNA targets. *PLoS Biol* 2004;2(11):e363
  85. Ramasamy S, Duraisamy S, Barbashov S, et al. The MUC1 and galectin-3 oncoproteins function in a microRNA-dependent regulatory loop. *Mol Cell* 2007;27(6):992-1004
  86. Yu W, Gius D, Onyango P, et al. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature* 2008;451(7175):202-6
  87. Lapidot M, Pilpel Y. Genome-wide natural antisense transcription: coupling its regulation to its different regulatory mechanisms. *EMBO Rep* 2006;7(12):1216-22
  88. He Y, Vogelstein B, Velculescu VE, et al. The antisense transcriptomes of human cells. *Science* 2008;322(5909):1855-7
  89. Belinsky SA. Gene-promoter hypermethylation as a biomarker in lung cancer. *Nat Rev Cancer* 2004;4(9):707-17
  90. Matsubayashi H, Canto M, Sato N, et al. DNA methylation alterations in the pancreatic juice of patients with suspected pancreatic disease. *Cancer Res* 2006;66(2):1208-17
  91. Schuebel KE, Chen W, Cope L, et al. Comparing the DNA hypermethylome with gene mutations in human colorectal cancer. *PLoS Genet* 2007;3(9):1709-23
  92. Laird PW. The power and the promise of DNA methylation markers. *Nat Rev Cancer* 2003;3(4):253-66
  93. Cairns P, Esteller M, Herman JG, et al. Molecular detection of prostate cancer in urine by GSTP1 hypermethylation. *Clin Cancer Res* 2001;7(9):2727-30

94. Kawakami K, Brabender J, Lord RV, et al. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J Natl Cancer Inst* 2000;92(22):1805-11
95. Issa JP. CpG island methylator phenotype in cancer. *Nat Rev Cancer* 2004;4(12):988-93
96. Shames DS, Girard L, Gao B, et al. A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. *PLoS Med* 2006;3(12):e486
97. Mizoshita T, Tsukamoto T, Inada KI, et al. Loss of MUC2 expression correlates with progression along the adenoma-carcinoma sequence pathway as well as de novo carcinogenesis in the colon. *Histol Histopathol* 2007;22(3):251-60
98. Copin MC, Buisine MP, Devisme L, et al. Normal respiratory mucosa, precursor lesions and lung carcinomas: differential expression of human mucin genes. *Front Biosci* 2001;6:D1264-75
99. Rakha EA, Boyce RW, Abd El-Rehim D, et al. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol* 2005;18(10):1295-304
100. Swartz MJ, Batra SK, Varshney GC, et al. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. *Am J Clin Pathol* 2002;117(5):791-6
101. Byrd JC, Bresalier RS. Mucins and mucin binding proteins in colorectal cancer. *Cancer Metastasis Rev* 2004;23(1-2):77-99
102. Chaturvedi P, Singh AP, Moniaux N, et al. MUC4 mucin potentiates pancreatic tumor cell proliferation, survival, and invasive properties and interferes with its interaction to extracellular matrix proteins. *Mol Cancer Res* 2007
103. Esteller M. Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. *Br J Cancer* 2006;94(2):179-83
104. Li Y, Liu D, Chen D, et al. Human DF3/MUC1 carcinoma-associated protein functions as an oncogene. *Oncogene* 2003;22(38):6107-10
105. Piacentini P, Donadelli M, Costanzo C, et al. Trichostatin A enhances the response of chemotherapeutic agents in inhibiting pancreatic cancer cell proliferation. *Virchows Arch* 2006;448(6):797-804
106. Van Seuningen I, Perrais M, Pigny P, et al. Sequence of the 5'-flanking region and promoter activity of the human mucin gene MUC5B in different phenotypes of colon cancer cells. *Biochem J* 2000;348(Pt 3):675-86.

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