Abstract
Transcriptional silencing of tumor suppressor genes, associated with DNA methylation, is a common epigenetic event in hematologic malignancies. Although DNA hypermethylation of CpG islands is well described in acute leukemias and myelodysplastic syndromes, much less is known of the specific methylation changes that commonly occur in follicular B cell lymphomas. Earlier methylation studies of follicular lymphoma involved only cell lines; however there is a growing literature of methylation changes in primary human FL samples. Published studies of primary follicular lymphoma specimens have demonstrated that: androgen receptor, SHP1, and death-associated protein kinase genes are commonly methylated. By contrast, the cyclin dependent kinase inhibitors p15, p16, and p57 are uncommon epigenetic events in follicular lymphoma. Methylation of cyclin dependent kinase inhibitors is more common in high grade lymphomas, and may be an important step in the progression and transformation of follicular lymphoma. Further methylation studies in follicular lymphoma should investigate the prognostic and therapeutic significance of these epigenetic changes and investigate methylation of other genes. Finally, reactivation of methylated tumor suppressor genes through the use of hypomethylating agents is a promising and novel approach to the treatment of indolent and transformed follicular lymphomas.

Background
Follicular lymphoma (FL), an indolent subgroup of non-hodgkin lymphomas, is a monoclonal lymphoid neoplasm arising from a malignant germinal center B lymphocyte. FL has an average annual incidence rate of 2.6 per 100,000 people and a median survival of 7.8 years [1,2].

Recent molecular studies have established that activation of various oncogenes and silencing of tumor suppressor genes is required for FL development and progression. Here we discuss published literature regarding transcriptional silencing associated with DNA methylation in FL.

In particular, we detail the genes known to be frequently methylated in FL, discuss their associated protein's function, and conclude with considerations for incorporating hypomethylating agents to reactivate methylated tumor suppressor genes as a novel therapeutic strategy for indolent and transformed FL.

Cancer genetics and epigenetics
Tumor suppressor genes
Tumor suppressor genes protect cells from undergoing malignant transformation. Tumor suppressor genes function by one of the following mechanisms: protect the genome from mutagenic events, impede dysregulated
progression through the cell cycle, induce apoptosis in cells that escape normal cell cycle controls, and inhibit cellular migration and metastasis. Classically, tumor suppressor genes have been described to acquire loss of function mutations or deletions leading to their inability to impede malignant transformation. Alternatively, epigenetic events, such as methylation, represent a distinct mechanism of tumor suppressor gene inactivation. Aberrant gene promoter methylation is associated with gene silencing and is functionally equivalent to a deleted gene. Gene silencing by DNA methylation has been considered to be permanent in non-embryonic cells, only reversible pharmacologically during cell division. Interestingly, new findings in lymphocytes may challenge this paradigm of irreversibility but have yet to be widely replicated nor specifically studied in FL [3].

**Promoter methylation down regulates transcription**

The promoter region of a gene is located upstream, at the 5’ end, from the transcription start site. Promoter regions of genes are more rich in cytosine-guanine dinucleotides than would be expected by chance alone, relative to the rest of the genome [4]. These cytosine guanine rich regions are referred to as CpG islands. Many mammalian genes have CpG islands in their promoter regions and are not methylated if the gene is to be transcribed within the cell. Methylation of cytosine residues in CpG islands, by DNA methyltransferase enzymes, is associated with transcriptional silencing.

Tumor suppressor gene methylation is a well recognized mechanism of oncogenesis in many tumor types. B-cell malignancies seem to be particularly susceptible to this phenomenon [5]. Although much of what is known about gene methylation in lymphoma has been derived from study of tumor cell lines, an increasing number of published studies have recently investigated primary human tumor samples. We review the current published literature of aberrant tumor suppressor gene methylation in primary human FL.

**DNA methylation analysis techniques**

Several methods to evaluate cytosine methylation are available and a complete review of these techniques is beyond the scope of this article. However, a succinct and thorough review of these methods is available [6]. The most commonly utilized methodologies for distinguishing methylated from unmethylated DNA all rely on DNA treatment with sodium bisulfite, which results in the conversion of all unmethylated cytosines to uracil, while leaving methylated cytosines unaltered. Two commonly utilized methods for quantification of DNA methylation are methylation specific polymerase chain reaction (MSP) and restriction enzyme-related polymerase chain reaction (ERP). Both methods require polymerase chain reactions (PCR) amplification of a specific locus within the CpG island of the gene of interest. MSP is a rapid and very sensitive technique to screen methylation where PCR primers designed to amplify either methylated or unmethylated bisulfite converted DNA are utilized; the intensities of methylated and unmethylated bands are analyzed by gel electrophoresis. The primary advantage of MSP is that it is thus far the most sensitive method and can detect 0.1% methylation. The disadvantages of this approach include: PCR bias (differential ability of methylated or unmethylated PCR primers to amplify gene product) and semi-quantitative nature. ERP utilizes methylation specific endonucleases to differentially cleave DNA fragments prior to undergoing PCR. ERP is not a widely used methodology due to problems with sensitivity.

**Commonly methylated genes in FL**

**Androgen receptor**

The androgen receptor gene, located on the X chromosome, encodes a member of the receptor group that binds and mediates the actions of androgens [7]. When bound and activated by an androgen, the androgen receptor enters the nucleus and acts as a ligand-dependent transcription factor to upregulate dependent gene transcription [7]. This pathway appears important in regulating lymphopoiesis. Thymus size normally decreases during puberty as a result of increased levels of sex hormones. By contrast, in castrated males thymic hypertrophy has been reported [8]. Whether from decreased androgen production or loss of receptor function, loss of the androgen signal leads to increased release of immature B cells from the bone marrow into the peripheral blood [9].

DNA promoter hypermethylation of the androgen receptor gene is a common finding in FL and other lymphomas. McDonald and colleagues reported 16/19 samples of T and B lymphomas positive for methylated androgen receptor genes [10]. A similar analysis of grade I and II FL found 25/26 samples tested were positive for methylation at the androgen receptor promoter region [11]. This consistent finding suggests that androgen receptor promoter methylation may be a common epigenetic phenomenon in FL pathogenesis.

**SHP1**

SHP1, also known as PTP1C, PTPN6, HCP, and SHPTP1, is a phosphotyrosine phosphatase that plays many important roles in regulating immune system cell differentiation and activation [12,13]. SHP1 acts as a growth inhibitor in B-cells by down regulating the intracellular effects of immunoglobulin binding thus requiring more receptor binding to initiate B-cell activation and proliferation [12,13]. Conversely, B lymphocytes with decreased SHP1 activity are more likely to proliferate and escape apoptosis [12,13]. Methylation of SHP1 promoter region appears...
common across a variety of lymphomas. Most FL samples, 32 of 33 assayed, were methylated at the SHP1 promoter region [14,15]. Additionally, SHP1 was methylated in 100% of plasmacytomas, 93% of diffuse large B cell lymphomas, 82% of MALT lymphomas, and 82% of mantle cell lymphomas [14,15]. Conversely, no promoter methylation of SHP1 was noted in 20 normal reactive lymph node samples [14]. Both the nearly uniform methylation of SHP1 in FL samples and the high rates across several lymphoma types suggest that down regulation of SHP1 by promoter hypermethylation may be an important step in lymphomagenesis.

**DAP kinase**

Death-associated protein kinase (DAPK) is a calcium-calmodulin-dependent serine/threonine kinase that participates in apoptosis [16]. DAPK was first demonstrated to participate in interferon-γ induced apoptosis [17]. Subsequently, DAPK has also been shown to contribute to tumor necrosis factor-α and Fas-induced apoptosis [18]. Blocking DAPK transcription in cells protects them from interferon-γ induced apoptosis but not the cell cycle inhibitory effects of interferon-γ [16]. Loss of DAPK expression due to gene hypermethylation has been demonstrated in bladder and renal cell carcinoma cell lines, immortalized B-cell lines, and primary, non-immortalized, B-cell lymphoma samples [19,20].

Methylation of DAPK may be a common epigenetic event in FL. In two published studies, twenty-five of twenty-nine (86%) FL samples were positive for aberrant DAPK methylation [5,21]. DAPK protein was not expressed in those lymphomas with DAPK methylation [5]. Similarly, in nine samples of transformed FL studied before and after transformation, 7/9 were found to have aberrant methylation of DAPK both before and after transformation [5]. Neither of the two unmethylated samples became methylated during the transformation [5]. These findings are consistent with the hypothesis that methylation of DAPK is a common, early epigenetic phenomenon of FL that allows these cells to escape the normal apoptosis of a non-antigen stimulated expanded b-cell population. However, there is also a correlation between increased DAPK methylation and increasing age as a potential confounding variable in such studies.

**Methylated cyclin dependent kinases inhibitors associated with FL transformation**

p16

p16, located on chromosome 9p21, is the most commonly altered gene in human malignancies [22]. p16 is also known by the following names: INK4B, INK4A, CDK4I, MTS1, and CDKN2A [22]. p16 is a 148 amino acid protein containing 4 ankyrin repeats [23]. p16 inhibits the phosphorylation of retinoblastoma protein and thereby impedes mitosis at G1-S transition of the cell cycle pathway [23]. By blocking unregulated cellular proliferation, p16 functions as an important tumor suppressor. The loss of p16 activity, either through gene mutation or promoter hypermethylation, is a common step in tumor development and progression.

Although homozygous deletion of p16 is common in numerous malignancies, it is uncommon in patients with newly diagnosed FL. Only two published studies have tested for p16 deletions in FL. Of twenty-nine FL cases tested, none showed the deletion [24,25]. However, one of nine patients with transformed FL did have a homozygous p16 deletion [25]. Little data exists examining p16 hypermethylation in FL. A combined analysis of published data showed aberrant p16 promoter methylation in five of sixteen FL cases [26,27]. Although not specifically reported as FL, another report of patients with "NHL-low grade" found one of eleven patients had tumors with detectible p16 promoter methylation [28].

However, p16 hypermethylation appears to be more frequent in lymphomas with a higher growth fraction. In two studies, fourteen of twenty-four high grade lymphoma samples were demonstrated p16 hypermethylation [27,28]. These findings suggest that p16 methylation may be an important epigenetic event in the progression and transformation of FL [25-27].

p15

p15, also known as INK4B, MTS2 and CDKN2B, is located 25 kilobases from the p16 gene and its protein shares significant areas of homologous amino acid sequences with p16 [29]. p15 inhibits cyclin dependent kinases 4 and 6 contributing to cell cycle arrest at the G1-S transition [30]. In contrast to p16, p15 expression is induced when cells are exposed to transforming growth factor-β (TGF-β) and may be essential to regulating the inhibitory cell cycle effects of this cytokine [29]. Similarly, the loss of p15 expression may contribute to TGF-β independent, dysregulated lymphopoesis. However, presently it is unclear whether p15 is an independent tumor suppressor gene, as most studies of primary tumor samples reporting p15 deletion have found simultaneous deletion of p16 also [31-33].

Methylation of the p15 promoter region has been reported in acute myeloid and lymphoblastic leukemias, multiple myeloma, and lymphomas [27,28,34,35]. Few studies have specifically detailed the histologic subtype of the lymphomas analyzed. Of those that have, ten of twenty-seven FL samples were found to have detectable p15 methylation [27,35]. Of the p15 methylated samples, 6/10 were also found to have p16 methylation [27,35]. The high concordance of p15 and p16 methylation makes it
difficult to determine the independent importance of either gene alone.

p57
p57, also known as KIP2 or CDKN1C, is a cyclin-dependant kinase inhibitor. The p57 gene is located on chromosome 11p15.5 [36]. p57 is a tight-binding inhibitor of several G1 cyclin/Cdk complexes and a negative regulator of cell proliferation. Mutations of p57 are implicated in sporadic cancers and Beckwith-Wiedemann syndrome suggesting that it is a tumor suppressor gene [37-39]. In a recent study, eight of eighteen FL samples (44%) had methylation of the p57 promoter region [40]. A similar proportion of p57 methylation is found in diffuse large B-cell lymphoma samples [40].

p14 is not commonly methylated
p14 protein, also known as p19ARF or CDKN2A, is also a cyclin-dependent kinase inhibitor known to induce cell cycle arrest at G1 and G2 [41]. p14 is encoded by three exons designated 1-β, 2, and 3 [41]. Exon 2 and 3 is shared with p16 although exon 1 is unique for each of the proteins [41]. Mice with homozygous deletion of p14 frequently develop numerous tumors including lymphomas [42]. Despite these interesting findings, Baur and colleagues found no methylation of the p14 promoter region 5' to exon 1-β in 56 lymphoma samples, which included 14 FL samples [27]. Therefore, hypermethylation of p14 does not appear to be a common epigenetic event in FL.

Methylation of genes with developing interest
GSTP1
GSTP1 is an important enzyme in the detoxification of mutagens [43,44]. Different polymorphisms of GSTP1 have been associated with the development of lymphoma, suggesting that GSTP1 may be important in the development and/or progression of lymphoma in humans [45]. Less active polymorphisms of GSTP1 confer less resistance to lymphoma development. In one study evaluating promoter methylation of GSTP1, ten of eighteen FL samples were found to have GSTP1 methylation [5]. Similarly, GSTP1 methylation was detected in six of eight hairy cell leukemia samples; by contrast, no methylation was detected in twelve chronic lymphocytic leukemia samples [5]. Further study in FL is warranted as the down regulation of GSTP1 may be an early event in lymphomagenesis.

IL-12R β2
Interleukin-12 is a pro-inflammatory cytokine produced by dendritic cells and phagocytes that promotes production of interferon-γ and acts synergistically with other cytokines to augment the proliferation and activity of T and NK cells [46]. The direct effects of IL-12 on B cells are not fully understood, but may act through the NFXB pathway in naïve B cells to produce interferon-γ [47]. The IL-12 receptor is composed of two subunits: β-1 and β-2 [48]. The β-1 subunit can also dimerize in the formation of IL-23 receptor, but the β-2 subunit is unique to the IL-12 receptor [46]. Although the β-2 subunit is normally expressed on the surface of B-lymphocytes, its expression has not been detected in various malignant B cell lines, suggesting its potential importance in malignant progression [49].

In a recent study, IL-12 receptor β-2 was not expressed in 12 FL samples tested [50]. Two of these samples were tested for DNA methylation of the IL-12 receptor β-2 gene, and both found to be methylated [50]. Results for mantle cell lymphoma, marginal zone lymphoma, and B cell chronic lymphocytic leukemia (CLL) were similarly methylated [50]. Incubation with 5-aza-2’-deoxycytidine, a demethylating agent, resulted in the re-expression of IL-12 receptor β-2 in FL and CLL cells [50]. Although the mechanism is not entirely clear, these data suggest IL-12 may serve as a tumor suppressor across a wide variety of B cell malignancies and methylation of IL-12 receptor β-2 gene may be a common step in the development of B cell malignancies.

Snk Plk2
Polo-like kinase 2 (Snk/Plk2) is a serine-threonine kinase that contributes to cells transitioning from G1 to S phase [51]. The gene is located at 5q12.1-q13.2 [52]. Although cells lacking this enzyme are more sensitive to paclitaxel induced cell death, it is unclear how this enzyme acts as a tumor suppressor [52]. Snk/Plk2 promoter region was found to be methylated in two of four FL samples in one study [53]. The significance of these findings and the impact of epigenetic regulation of Snk/Plk2 on lymphomagenesis still need to be further explored.

GADD45-γ
GADD45 proteins α, β, and γ, are induced by DNA damage and extrinsic stressors [54]. GADD45-γ exerts at least part of its influence by inhibiting the Cdk1/cyclinB1 complex and blocking the S and G2-M cell cycle transition [55]. Tumor cell lines previously lacking GADD45-γ expression that were transfected with GADD45-γ showed significant reduction in colony formation and some cases with frank apoptosis and no culture growth [56]. Gene methylation of GADD45-γ has only rarely been reported in primary FL samples. In one recent study, although 11/13 non-hodgkin lymphoma cell lines were GADD45-γ methylated, only 1/6 primary FL samples were found to be methylated [56]. The apparent discordance between cell lines and primary patient samples will require further evaluation to better estimate the prevalence of GADD45-γ methylation in FL.
Annexin 1

Annexin 1 is a calcium and phospholipid binding protein that may participate in both spontaneous and stimulated apoptosis in some cell lines [57]. Although annexin 1 is expressed in normal adenoid B-cells, it is not detected in FL cell lines or Burkitt lymphoma cell lines [58]. The annexin 1 gene was analyzed in Burkitt lymphoma cell lines and found to be structurally intact but methylated [58]. Annexin 1 was re-expressed when these Burkitt lymphoma cells were cultured with 5-aza-2'-deoxycytidine suggesting that aberrant annexin 1 methylation explains decreased annexin 1 expression in FL cells. [58]. Additional studies on patient FL samples are needed to better clarify whether annexin 1 methylation is a common epigenetic event in FL.

Treatment strategies

With combination chemotherapy and rituximab, FL patients may have long remissions. Unfortunately, relapses are part of the natural history of FL. With the exception of treatment with allogeneic bone marrow transplant or stage I disease that is irradiated or resected, FL is generally considered incurable. Most patients will experience multiple relapses of their lymphoma. Patients generally have a shorter response to chemotherapy with each episode of relapsing disease. Further, patients are at risk for their lymphoma transforming from an indolent FL to a more aggressive and treatment refractory lymphoma as the disease progresses.

Hypomethylating agents

Azacitidine (5-azacytidine) [59] and decitabine (5-aza-2'-deoxycytidine) [60] are two hypomethylating medications approved by the United States Food and Drug Administration for the treatment of myelodysplastic syndromes. Other similar compounds have been described but are not employed clinically in the United States [61]. At concentrations above those required to achieve demethylation, these cytidine analogues have direct cytotoxicity by inhibiting DNA synthesis [59,62]. However, clinically these agents are believed to be incorporated into replicating DNA and inhibit DNA methyltransferases (DNMT) [59,62]. During normal cellular replication, the methylation status of each gene is well conserved from the parent to daughter cells. When DNA methyltransferases are inhibited, the newly replicated DNA cannot be methylated by DNMT, allowing transcription and translation of the associated gene. Although beyond the scope of this article, a full discussion of the mechanism of action of this class of medications is available [63]. There is a theoretical concern that treatment with hypomethylating agents may induce the expression of silenced oncogenes or allow translocation of transposable elements within the genome [64]. However, in clinical trials of azacitidine in patients with myelodysplastic syndrome, progression to leukemia was reduced with treatment suggesting that if present, the mutagenic risk of hypomethylation is smaller than the therapeutic benefit in that clonal malignancy [65].

Strategies for therapeutic demethylation

We have reviewed the growing body of clinical data detailing genes known to be hypermethylated in FL. Although no clinical trial has specifically investigated hypomethylating agents in patients with FL, individual patients with various lymphomas have been included in early development trials of these agents [66-68]. Some partial responses were reported in these single agent trials, although no documented response lasted longer than seven months and the type of lymphomas are not well described [66-68]. In the future, four unique settings exist to investigate hypomethylating agents in FL: i. refractory disease; ii. transformed lymphoma; iii. alternative to traditional cytotoxic chemotherapy; iv. adjuvant to induction cytotoxic chemotherapy.

Patients with refractory cancers are often considered for early development trials as they have already failed standard therapies. Given lymphoma patients short duration of response in phase I trials of DNMT inhibitors, we expect DNMT inhibitors will be most active when incorporated into a multi-drug regimen. Additionally, FL transformation is associated with aberrant methylation of cyclin dependent kinase inhibitors and therapeutic reversal should be explored in this setting. Another appealing strategy is to treat FL patients without cytotoxic therapies. If the hypomethylating agents can induce the expression of androgen receptor, DAPK, and IL-12 receptor β-2, then concurrent treatment with their respective ligands may be investigated clinically. Finally, if a clinical benefit is demonstrated in these settings, DNMT inhibitors could be evaluated as an adjuvant to FL induction chemotherapy regimens.

Conclusion

Androgen receptor, SHP1, and DAPK are commonly methylated genes in FL. We have described a growing list of additional genes that may be frequently methylated in FL and are under investigation. Additionally, methylation of cyclin dependent kinase inhibitors p16, p15, and p57 may be associated with transformation of FL to a more aggressive lymphoma in some patients. More research is needed to identify epigenetic events important to lymphomagenesis and progression as well as identifying prognostic relevance. Clinical trials to develop therapeutic strategies with hypomethylating agents are needed. Preclinical data with hypomethylating agents in FL are promising and offer a new paradigm for clinical exploration in the treatment of patients with FL.
Table 1: Methylated genes in primary follicular lymphoma samples

| Gene                  | # of Methylated Samples (%) | Method of analysis | Gene function                                                                 | Reference |
|-----------------------|----------------------------|--------------------|--------------------------------------------------------------------------------|-----------|
| Androgen receptor     | 25/26 (96)                 | MSP                | Ligand-dependent transcription factor                                           | [11]      |
| SHP1                  | 32/33 (97)                 | MSP                | Inhibits intracellular effects of surface immunoglobulin binding in B lymphocytes | [14, 15]  |
| Death-associated protein kinase | 25/29 (86) | MSP                | Involved in interferon-γ, tumor necrosis factor cytokines, and Fas-ligand induced apoptosis | [5, 21] |
| p16                   | 5/16 (31)                  | ERP                | Cyclin dependent kinase inhibitor                                              | [26, 27]  |
| p15                   | 10/27 (37)                 | ERP                | Cyclin dependent kinase inhibitor                                              | [27, 35]  |
| p57                   | 8/18 (44)                  | MSP                | Cyclin dependent kinase inhibitor                                              | [40]      |
| GIST1                 | 10/18 (56)                 | MSP                | Detoxify mutagens                                                             | [5]       |
| IL-12 receptor β-2    | 2/2 (100)                  | MSP                | Forms heterodimer with IL-12R1 to form IL-12 receptor                          | [50]      |
| Snk/plk2              | 2/4 (50)                   | MSP                | DNA damage response and cell growth arrest                                   | [53]      |
| GADD45-γ              | 1/6 (17)                   | MSP                | DNA damage response and cell growth arrest                                   | [56]      |

Abbreviations. MSP: Methylation specific PCR; ERP: Restriction enzyme-related PCR

References

1. Groves FD, Linet MS, Travis LB, Devesa SS. Cancer surveillance series: surveillance for cancer of the lymphoid and hematopoietic tissues: histologic subsite incidence in the United States from 1973 through 1995. J Natl Cancer Inst 2000, 92(15):1240-1251.

2. Swenson WT, Woolridge JE, Lynch CF, Forman-Hoffman VL, Chrischilles E, Link BK. Improved survival of follicular lymphoma patients in the United States. J Clin Oncol 2005, 23(22):5019-5026.

3. Kersh EN, Fitzpatrick DR, Murali-Krishna K, Shires J, Speck SH, Boss JM, Ahmed R. Rapid demethylation of the IFN-gamma gene occurs in memory but not naive CD8 T cells. J Immunol 2006, 176(7):4083-4093.

4. Santini V, Kantarjian HM, Issa JP. Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications. Ann Intern Med 2001, 134(7):573-586.

5. Rossi D, Capello D, Gloghini A, Franceschetti S, Pauli M, Bhatia K, Saglio G, Vitolio U, Pileri SA, Esteller M, Carbono A, Gaidano G. Aberrant promoter methylation of multiple genes throughout the clinicopathologic spectrum of B-cell neoplasia. Haematologica 2004, 89(3):154-164.

6. Oakeley EJ. DNA methylation analysis: a review of current methodologies. Pharmacol Ther 1999, 84(3):389-400.

7. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev 2002, 23(2):175-200.

8. Eideg C, Garrett JJ. Studies of the regulatory effects of sex hormones on antibody formation and stem cell differentiation. J Exp Med 1972, 136(5):1098-1116.

9. Ellis TM, Moser MT, Le PT, Flanigan RC, Kwon ED. Alterations in peripheral B cells and B cell progenitors following androgen ablation in mice. Int Immunol 2001, 13(4):553-558.

10. McDonald HL, Gasscoyne RD, Horsman D, Brown CJ. Involvement of the X chromosome in non-Hodgkin lymphoma. Genes Chromosomes Cancer 2000, 28(3):246-257.

11. Yang H, Chen CM, Yan P, Huang TH, Shi H, Burger M, Nimnich I, Maier S, Berlin K, Caldwell CW. The androgen receptor gene is preferentially hypermethylated in follicular non-Hodgkin’s lymphomas. Clin Cancer Res 2003, 9(1):4034-4042.

12. Giri K, Kozlowski M, Cambier JC, Mills GB, Siminovitch KA. Identification of the tyrosine phosphate PTPC1 as a B cell antigen receptor-associated protein involved in the regulation of B cell receptor signaling. J Exp Med 1995, 181(6):2077-2084.

13. Cyster JG, Goodnow CC. Protein tyrosine phosphatase 1C negatively regulates antigen receptor signaling in B lymphocytes and determines thresholds for negative selection. Immunity 1995, 2(1):13-24.

14. Koyama M, Oka T, Ouchida M, Nakatani Y, Nishiiuchi R, Yoshino T, Hayashi K, Akagi T, Seino Y. Activated proliferation of B-cell lymphomas/leukemias with the SHP1 gene silencing by aberrant CpG methylation. Lab Invest 2003, 83(12):1849-1858.

15. Chin CS, Wong KY, Loong F, Srivastava G. SOCS1 and SHP1 hypermethylation in mantle cell lymphoma and follicular lymphoma: implications for epigenetic activation of the Jak/STAT pathway. Leukemia 2004, 18(2):356-358.

16. Deiss LP, Feinstein E, Berissi H, Cohen O, Kimchi A. Identification of a novel serine/threonine kinase and a novel 15.4-kDa protein as potential mediators of the gamma interferon-induced cell death. Genes Dev 1995, 9(1):15-30.

17. Deiss LP, Kimchi A. A genetic tool used to identify thiorodoxin as a mediator of a growth inhibitory signal. Science 1991, 252(5002):117-120.

18. Cohen O, Inbal B, Kissil JL, Raveh T, Berissi H, Spivak-Kroizman T, Feinstein E, Kimchi A. DAP-kinase participates in TNF-alpha and Fas-induced apoptosis and its function requires the death domain. J Cell Biol 1999, 146(1):141-148.

19. Katsanellenbogen RA, Baylin SB, Herman JG. Hypermethylation of the DAP-kinase Cpg island is a common alteration in B-cell malignancies. Blood 1999, 93(12):4347-4353.

20. Kissil JL, Feinstein E, Cohen O, Jones PA, Tsai YC, Knowles MA, Eydmann ME, Kimchi A. DAP-kinase loss of expression in various carcinoma and B-cell malignancy cell lines: possible implications for role as tumor suppressor gene. Oncogene 1997, 15(4):403-407.

21. Nakatsu S, Takakuwa T, Tomita Y, Hoshida Y, Nishii M, Yamaguchi M, Nishii K, Yang WI, Aozasa K. Hypermethylation of death-associated protein (DAP) kinase Cpg island is frequent not only in B-cell but also in T- and Natural killer (NK)/T-cell malignancies. Cancer Sci 2003, 94(1):87-91.

22. Hirama T, Koefler HP. Role of the cyclin-dependent kinase inhibitors in the development of cancer. Blood 1995, 86(3):841-854.

23. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/Cdk4. Nature 1993, 366(6456):704-707.

24. Stranks G, Height SE, Mitchel L, Jadayel D, Yuille MA, De Lord C, Clutterbuck RD, Treleaven JG, Powles RL, Nacheva E, et al. Deletions and rearrangement of CDKN2 in lymphoid malignancies. Blood 1995, 85(4):893-901.

25. Pinyol M, Cabo F, Bea S, Jares P, Nayach I, Fernandez PL, Montserrat E, Cardesa A. Campo E. p16(INK4a) gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of non-Hodgkin’s lymphomas. Blood 1998, 91(8):2977-2984.

26. Villanueva R, Sanchez-Beato M, Martinez JC, Saiz AI, Martinez-Delgado B, Garcia JF, Matos HS, Sanchez-Verde L, Benitez J, Martinez P, Piris MA. Loss of p16(INK4a) protein expression in non-Hodgkin’s lymphomas is a frequent finding associated with tumor progression. Am J Pathol 1998, 153(3):887-897.

27. Bar AS, Shaw P, Burri N, Delacretaz F, Bozeman FT, Chaubert P. Frequent methylation silencing of p15(INK4b) (MTS2) and p16(INK4a) (MTS1) in B-cell and T-cell lymphomas. Blood 1999, 94(5):1773-1781.

28. Herman JG, Civan CI, Issa JP, Collector MI, Starkis SJ, Baylin SB. Distinct patterns of inactivation of p15INK4B and p16INK4A.
characterize the major types of hematological malignancies. Cancer Res 1997, 57(5):837-841.

29. Trinchieri G, Bel E, Fallall T: IL1NK4B is a potential effector of TGF-

beta-induced cell cycle arrest. Nature 1994, 371(6494):257-261.

30. Kamb A, Zbar B, Agranoff B, Anderson G, Artandi S, Balmain A, Bigner D, Bird A, Bigner D, Bova G, Bristow R, et al.: Genetic instability and the development of cancer. Science 1994, 263(5157):436-440.

31. Jen J, Harper JW, Shigematsu S, Sklar P, Squire J: Silencing of the p16 gene in human tumors. Cancer Res 1994, 54(12):6335-6338.

32. Theiler C, Lin D, Todd T, Bhatia S, Stockert E, Day RS, Johnson BE, Skolnick MH: A cell cycle regul-ator potentially involved in genesis of many tumor types. Science 1994, 263(5157):436-440.

33. Jen J, Harper JW, Shigematsu S, Sklar P, Squire J: Silencing of the p15 gene in human tumors. Cancer Res 1994, 54(12):6335-6338.

34. Washimi O, Nagatake M, Osada H, Ueda R, Koshikawa T, Seki T, Takahashi T, Takahashi T: In vivo occurrence of p16 (MTS1) and p15 (MTS2) alterations preferentially in non-small cell lung cancers. Cancer Res 1995, 55(3):514-517.

35. Freier J, Cayuela JM, Berkley J, Sigaux F: Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) displacing frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. Blood 1994, 84(12):4038-4044.

36. Ng MH, Chung YF, Lo KW, Wickham NW, Lee JC, Huang DP: Frequent homozygous deletions in primary cells from T but not from B-cell lineage acute lymphoblastic leukemias. Blood 1994, 84(12):4038-4044.

37. Freier J, Cayuela JM, Berkley J, Sigaux F: Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) displacing frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. Blood 1994, 84(12):4038-4044.

38. Martinez-Delgado B, Robledo M, Agran E, Osorio A, Garcia MJ, Freier J, Cayuela JM, Berkley J, Sigaux F: Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) displacing frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. Blood 1994, 84(12):4038-4044.

39. Martinez-Delgado B, Robledo M, Agran E, Osorio A, Garcia MJ, Freier J, Cayuela JM, Berkley J, Sigaux F: Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) displacing frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. Blood 1994, 84(12):4038-4044.