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Epigenetics wins over genetics: induction of differentiation in tumor cells

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Malignant cells are genetically abnormal, but can the malignant phenotype revert to a non-malignant phenotype without correcting these genetic abnormalities? It has been found that this reversion can be achieved by reprogramming tumor cells by epigenetic changes induced by differentiation. The epigenetic suppression of malignancy by inducing differentiation bypasses the genetic abnormalities in tumor cells. Studies with myeloid leukemic cells have shown that some leukemic cells can be induced to differentiate by cytokines that control normal hematopoiesis, and that myeloid leukemic cells resistant to normal cytokines can be induced to differentiate by compounds that use alternative differentiation pathways. The epigenetic reprogramming of tumor cells by inducing differentiation has also been found with other types of tumors and can be used for tumor therapy. By this reversion of the malignant to non-malignant phenotype, epigenetics wins over genetics.

Key words: cytokines / differentiation / epigenetic suppression of malignancy / hematopoiesis / leukemia / tumor therapy

Introduction

All cells in the body are descendents of a fertilized egg and in almost all cases contain the same set of genes. But there is a great diversity of normal cell types that carry out a variety of specific functions. These specific properties are determined by activating expression or repression of sets of genes by epigenetic changes. Such epigenetic events reprogram the genome in normal development into different types of differentiated somatic cells.[1,2] The origin of malignancy involves genetic changes in the DNA.[3,4] This raises the question whether malignant cells can revert back to cells that again show normal growth control.[5] Studies with sarcomas have shown that non-malignant cells can be derived from malignant cells[6] by segregation of specific chromosomes.[5] Hybridization between different cell types has also shown that non-malignant cells can be derived from malignant cells by specific chromosome changes.[3,7] The appropriate genetic changes either with or without cell hybridization can thus result in the reversion of malignancy. But can the malignant phenotype revert to a non-malignant phenotype without correcting the genetic abnormalities in malignant cells? We will discuss how this can be achieved by epigenetic changes induced by differentiation. Using hematopoiesis and leukemia as a model system,[5,8] we will also discuss the application of this conclusion to other types of tumors and to tumor therapy.

Epigenetic control of gene expression in differentiation

Cell differentiation requires cell type specific silencing of some genes and activation of other genes. The major change that leads to gene silencing in differentiation is methylation of DNA at CpG sites. This methylation prevents the binding of transcription factors to CpG dinucleotides in the gene promoter region.[9,10] Methylated DNA preferentially associates with deacetylated histones.[11] This preferential association is mediated by methyl cytosine binding proteins of the MBD and MeCP families that recruit transcriptional repressor protein complexes containing histone deacetylases (HDACs)[12] and histone methylases.[13,14] Histone methylation can trigger DNA methylation.[15] Gene silencing by DNA methylation[16] can also be induced by enhancing DNA methyl transferase activity.[17]

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Demethylation of DNA induces activation of gene expression in differentiation.[18–22] This can involve inhibition of DNA methyl transferase activity[2] or activation of DNA demethylase(s).[23] Structural chromatin changes, increased DNase I sensitivity and increased histone acetylation, can also lead to activation of gene expression.[21,24,25] Another mechanism for gene activation involves acetylation of transcription factors such as MyoD[26] and SCL/TAL1[27] by the histone acetyl transferase (HAT) P/CAF. Acetylation of MyoD and SCL/TAL1 increases their binding to DNA, reduces association with gene-silencing HDAC containing complexes and activates gene expression. In some tumors, there is DNA hypomethylation and concomitant CpG island hypermethlation[28–30] as well as abberant regulation of HATs and HDACs.[31,32]

What are the external inducers that induce the epigenetic changes for gene silencing and gene activation in normal differentiation, and can these inducers also epigenetically induce differentiation in malignant cells and reprogram the malignant to a non-malignant phenotype? To identify such inducers, we have studied, as a model system, the differentiation of different cell lineages from stem cells in hematopoiesis, and the ability of these normal inducers to induce differentiation in leukemia.

Cytokine control of stem cell differentiation in normal hematopoiesis

The formation of different types of blood cells, hematopoiesis, is essential for the development and survival of a normal individual. New blood cells belonging to different cell lineages are formed from stem cells during embryogenesis and throughout the lifetime of the adult. Identification of the external inducers that control normal blood cell development makes it possible to answer questions about the regulation of normal hematopoiesis and the origin and treatment of hematological diseases including leukemia. This became a reality about four decades ago when we established the first cell culture system in which normal hematopoietic cells, in the presence of feeder cells, could be cloned and made to develop to different cell lineages.[33] The concluding sentence of the first paper stated "The described cultures thus seem to offer a useful system for a quantitative kinetic approach to hematopoietic cell formation and for experimental studies on the mechanism and regulation of hematopoietic cell differentiation".[33] We then showed that the inducers of these hematopoietic cell clones are secreted by the feeder cells and are present in conditioned medium produced by the feeder cells.[34–36] This system, which was first established for myeloid cells, allowed the identification, purification and gene cloning of the cytokines that regulate hematopoiesis.[8,37]

The first inducer of normal macrophage and granulocyte colony formation identified in conditioned medium[35,36] was called *mashran gm* from the Hebrew word meaning to send forth with the initials for granulocytes and macrophages.[38] This and other colony-inducing cytokines were then renamed macrophage and granulocyte inducers (MGI)[39] and MGI-type 1 (MGI-1) and are now called colony-stimulating factors (CSFs)[37,40] and one cytokine is called interleukin-3 (IL-3).[41] Of the four different CSFs initially identified, one (M-CSF), induces the development of clones with macrophages, another (G-CSF), clones with granulocytes, the third (GM-CSF), clones with granulocytes, macrophages, or both macrophages and granulocytes, and the fourth (IL-3), clones with macrophages, granulocytes, eosinophils, mast cells, erythroid cells, or megakaryocytes. Later studies identified other cytokines that act on cells of the myeloid lineage including stem cell factor (SCF),[42] Flt3/Flk2 ligand (FL)[43] and thrombopoietin (TPO),[44] some of which can synergize with different CSFs. G-CSF is now used in the clinic to repair irradiation and chemotherapy associated suppression of normal hematopoiesis in cancer patients, to stimulate normal granulocyte development in patients with infantile congenital agranulocytosis, and to induce migration of hematopoietic stem cells from the bone marrow to peripheral blood for stem cell transplantation.[5,8] At appropriate *in vivo* locations, hematopoietic stem cells can differentiate into glial cells,[45] skeletal muscle,[46] liver cells[47] and epithelial cells.[48] Hematopoietic stem cells thus show considerable plasticity and can also reprogram their gene expression in response to signals produced at sites such as regenerating tissues, which presumably have the right combination of cytokines.

The development of clones with terminally differentiated, non-dividing, mature cells such as granulocytes and macrophages from single precursor cells requires induction of cell multiplication and differentiation associated with the arrest of cell multiplication. How can one explain the ability of a cytokine to transmit signals that activate these distinct and apparently contradicting processes in the same cell? We suggested that there is a cytokine cascade in the signalling pathways for multiplication and differentiation induced

by the CSFs. We therefore looked for a cytokine that acts as a myeloid cell differentiation inducer but does not have CSF activity, and found such a cytokine which we called MGI-2.[5,8] We showed that MGI-2 is identical to interleukin-6 (IL-6)[49] and it was suggested that there are presumably other hematopoietic cell differentiation inducing cytokines. Studies on myeloid leukemic cells have identified other differentiation inducing cytokines with no CSF activity, including leukemia inhibitory factor (LIF).[50,51] Another cytokine, interleukin-1 (IL-1), also induced differentiation in some clones of myeloid leukemic cells, and this is mediated by induction of IL-6.[52] IL-6 and IL-1 induce differentiation without apparently inducing cell multiplication in normal myeloid precursors.[53] Oncostatin M (OSM) and interleukin-11 (IL-11), which use the same cell surface signal transducing protein, gp130, that is used by IL-6 and LIF also induce myeloid differentiation[54] without CSF activity.

The existence of CSFs and distinct differentiation inducing cytokines led to the suggestion, that CSFs which induce growth and non-CSF cytokines which induce differentiation act in a cytokine cascade that ensures effective coupling of growth and differentiation.[5,8,37,55] This was confirmed by showing that all four CSFs, GM-CSF, G-CSF, M-CSF and IL-3, can induce in normal hematopoietic precursors, the production of IL-6 which does not induce the formation of colonies but can induce myeloid precursor cells to differentiate.[5,8,37,55] This cytokine cascade is part of a network that includes other cytokines.[5,8,56] Cytokine activation during differentiation in myeloid and lymphoid cells is mediated by DNA demethylation of cytokine genes.[20,21] Cytokines such as IL-1, IL-3, IL-6 and G-CSF induce expression of lysozyme during myeloid cell differentiation by demethylation of the lysozyme gene[19] and G-CSF induces histone acetylation in the myeloid specific gene myeloperoxidase.[24] IL-6 can also induce expression of DNA methyltransferase,[17] which can mediate silencing of certain genes during differentiation.

Reprogramming of gene expression in myeloid leukemic cells by inducing differentiation: reprogramming by cytokines

Leukemic and other malignant cells have abnormal developmental programs. These abnormalities are associated with genetic changes in the DNA in the form of various chromosomal aberrations, loss of suppressor genes and activation of oncogenes.[3,4] The existence of genetic changes in tumors raises the question whether genetically abnormal tumor cells can be epigenetically reprogrammed to regain normal behavior. Using myeloid leukemic cells as a model system, we first examined whether malignancy in these cells can be suppressed by inducing differentiation with normal cytokines.[8,37,57,58] We found that there are myeloid leukemic cells that can be induced to differentiate to non-dividing mature granulocytes and/or macrophages by adding different cytokines including IL-6 (Figure 1), IL-1, GM-CSF, G-CSF and IL-3.[8,37,56,58,59] It was then shown that differentiation can be induced in some myeloid leukemic cells by LIF, OSM, IL-11[54] and TNF.[60] These results show that there are myeloid leukemic cells that can be reprogrammed by normal cytokines to behave again like non-malignant cells. Different clones of myeloid leukemic cells can have different blocks in the ability to be induced to undergo differentiation by cytokines.[61,62] As described in the previous section, cytokines can regulate differentiation by epigenetic changes.

In vivo studies have shown that normal differentiation of myeloid leukemic cells can be induced not only in culture but also *in vivo*.[63,64] After injection of mouse myeloid leukemic cells into fetuses, leukemic cells were shown to participate in normal hematopoietic differentiation to mature granulocytes and macrophages in apparently healthy adult animals.[65,66] It has also been shown that mature granulocytes in human myeloid leukemia patients can be derived from leukemic cells.[67] The development of leukemia was inhibited in mice inoculated with leukemic cells by increasing the amount of differentiation inducing cytokine, either by injecting it or by injecting a compound that increased its production by cells in the body.[5,8,37,57,68,69] The finding that myeloid leukemic cells can be induced to differentiate to mature cells *in vitro* and *in vivo* by differentiation inducing cytokines and in doing so have lost their leukemogenicity *in vivo*, created the basis for differentiation therapy of leukemia.[8,70] The ability of the leukemic cells to be induced to differentiate by cytokines and regain normal growth control was not due to correction of their chromosomal abnormalities.[71,72] These studies indicate that an abnormal developmental program in myeloid leukemic cells can be reprogrammed epigenetically by appropriate differentiation inducing cytokines. The epigenetic suppression of malignancy by inducing differentiation bypasses the genetic abnormalities in malignant cells.[71,72]

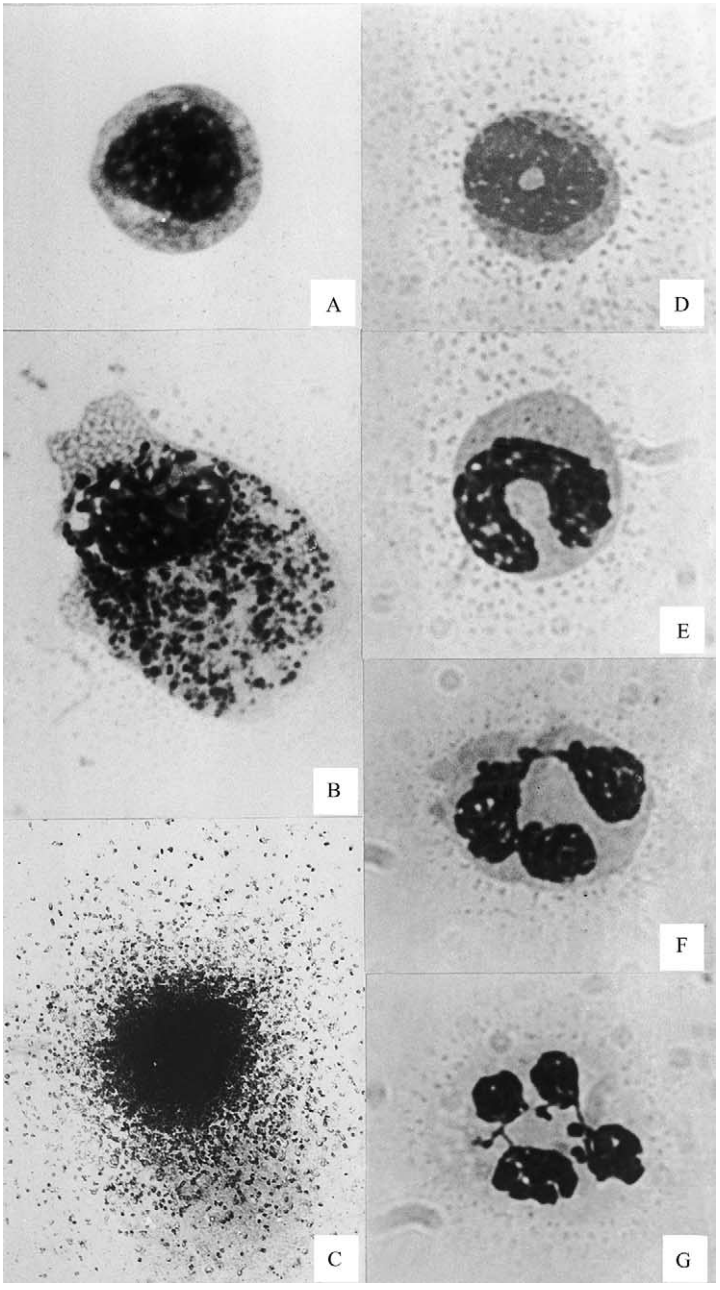

Figure 1. Epigenetic differentiation of genetically abnormal myeloid leukemic cells to non-malignant mature macrophages and granulocytes by IL-6: (A) leukemic cell; (B) macrophage; (C) colony with macrophages; (D–G) stages in differentiation to granulocytes.[59]

Reprogramming of gene expression in myeloid leukemic cells by inducing differentiation: reprogramming by other compounds

Studies with a variety of compounds, other than normal hematopoietic cytokines, have shown that various compounds induce differentiation in myeloid leukemic cells.[70] Various chemicals also induce differentiation in erythroleukemic cells.[73] For myeloid leukemic cells, these include compounds that are used today in cancer chemotherapy, such as cytosine arabinoside, methotrexate and others, irradiation and glucocorticoid hormones. At high doses, irradiation and the compounds used in cancer chemotherapy

kill cells by inducing apoptosis, whereas at low doses they induce differentiation. The compounds are not equally active on the same leukemic clone.[70,74] Differentiation induced by glucocorticoid hormones is presumably mediated by their ability to cause chromatin remodeling as well as by the association of ligand-bound glucocorticoid receptors with transcriptional co-activators some of which have HAT activity.[75] Some compounds can also induce differentiation in clones that are not induced to differentiate by a hematopoietic cytokine, and in some of these clones induction of differentiation requires combined treatment with different compounds.[74] In addition to chemotherapeutic compounds, radiation and steroids, other compounds that can induce differentiation in myeloid leukemic cells include insulin, bacterial lipopolysaccharide, tumor promoting phorbol esters[70,74] and retinoic acid (RA).[76] The ability of RA to induce terminal differentiation of human promyelocytic leukemia cells carrying the PML/RARα fusion protein is now used clinically in the therapy of this leukemia,[76] showing the successful application of the concept of differentiation therapy in the clinic.

Unliganded RARα and even more so PML/RARα fusion protein, block myeloid differentiation by recruiting DNA methyltransferases[77] and HDAC containing complexes[31,78] that repress transcription of target genes. Binding of RA results in degradation of the PML/RARα fusion protein which causes promoter demethylation,[77] relieves the HDAC mediated repression and allows differentiation to occur.[77,78] Arsenic trioxide can relieve recruitment of the transcriptional co-repressor SMRT to PML/RARα[79] and also causes degradation of the PML/RARα fusion protein.[78] This degradation results in induction of differentiation[78,80] and induces remission in PML/RARα promyelocytic leukemia patients.[80] Since the repression of gene expression by PML/RARα is mediated, at least in part, by HDAC containing complexes, inhibitors of HDAC activity[81] can relieve the block in differentiation by PML/RARα. HDAC inhibitors also enhance the ability of RA to relieve transcriptional repression and the differentiation block imposed by PML/RARα.[82] In leukemic cells which are only weakly responsive or nonresponsive to RA alone, the combination of HDAC inhibitors and RA relieves transcriptional repression and the differentiation block imposed by the fusion proteins PLZF–RARα[82] and AML1/ETO.[83] Inhibition of DNA methyltransferase by 5-aza-2′-deoxycytidine enhances differentiation of myeloid leukemic cells by RA and Vitamin D3 analogs even in cells that do not differentiate with RA or Vitamin D3 alone.[84] It is therefore possible that all myeloid leukemic cells which are no longer susceptible to the normal hematopoietic cytokines by themselves can be induced to differentiate by the appropriate combination of compounds. The combination of HDAC inhibitors and DNA methylation inhibitors synergistically activate transcription of hypermethylated genes.[85] It will be interesting to determine whether this combination may be an effective differentiation treatment in cells that resist other treatments. The experiments with myeloid leukemic cells have shown that there are different pathways of gene expression for inducing differentiation, and that genetic changes which suppress induction of differentiation by one compound need not affect differentiation by another compound using alternative pathways[74,86] (Figure 2).

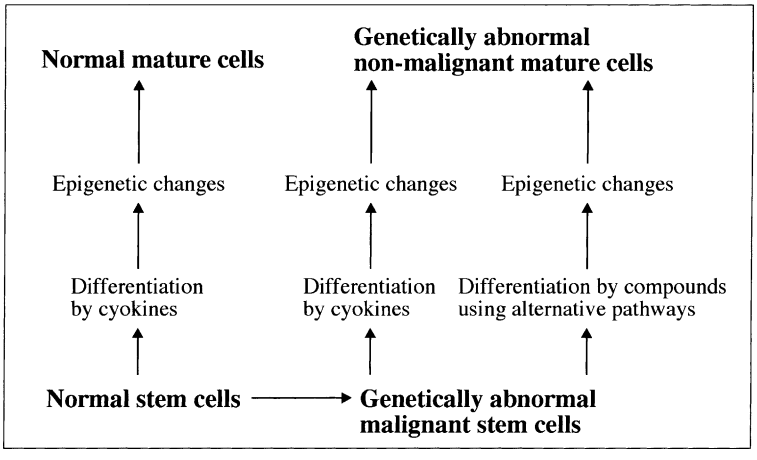

Figure 2. Epigenetic suppression of malignancy by inducing differentiation. Malignant cells are genetically abnormal. The malignant phenotype can be epigenetically reprogrammed to a non-malignant phenotype by inducing differentiation.

Reprogramming of gene expression by inducing differentiation in other types of tumors

The principles outlined above on differentiation of myeloid leukemic cells with cytokines and some other compounds also apply to other tumors. Melanoma cells can be induced to differentiate by a combination of interferon β and mezerein,[87] interferon γ and the phorbol ester TPA or interferon γ and RA.[88] Human prostate cancer cells differentiate by treatment with IL-6 or cAMP elevating compounds and even better when both were added together.[89] Colon carcinoma cells differentiate by Vitamin D3,[90] TPA[91] or brefeldin A.[92] Human neuroblastoma cells differentiate by RA and herbimycin and even better by their combination[93] and prostate cancer cells[94] and breast cancer cells[95] differentiate with HDAC inhibitors. The use of HDAC inhibitors *in vivo* in mice has shown that these compounds inhibit the growth of leukemia and some other types of tumors with little toxicity to the mice.[81] Both HDAC inhibitors[81] and RA analogs (retinoids) that bind to RAR or RXR[96] are currently being tested clinically in therapy of leukemia and other tumors.

The results with myeloid leukemia and other tumors, which are all genetically abnormal, have shown that tumor cells can be epigenetically reprogrammed to a non-malignant phenotype by inducing differentiation. The epigenetic suppression of malignancy by inducing differentiation bypasses the genetic abnormalities in tumor cells and can be used in tumor therapy. In this reprogramming of a malignant to a non-malignant phenotype, epigenetics wins over genetics.

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References

1. Surani MA (2001) Reprogramming of genome function through epigenetic inheritance. Nature 414:122–128
2. Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. Science 293:1089–1093
3. Klein G (1981) The role of gene dosage and genetic transpositions in carcinogenesis. Nature 294:313–318
4. Rowley JD (1998) The critical role of chromosome translocations in human leukemias. Annu Rev Genet 32:495–519
5. Sachs L, (1995) The adventures of a biologist: prenatal diagnosis, hematopoiesis, leukemia, carcinogenesis and tumor suppression. Adv Cancer Res 66:1–40
6. Rabinowitz Z, Sachs L (1968) Reversion of properties in cells transformed by polyoma virus. Nature 220:1203–1206
7. Harris H, Miller OJ, Klein G, Worst P, Tachibana T (1969) Suppression of malignancy by cell fusion. Nature 223:363–368
8. Sachs L (1996) The control of hematopoiesis and leukemia: from basic biology to the clinic. Proc Natl Acad Sci USA 93:4742–4749
9. Weih F, Nitsch D, Reik A, Schutz G, Becker PB (1991) Analysis of CpG methylation and genomic footprinting at the tyrosine aminotransferase gene: DNA methylation alone is not sufficient to prevent protein binding *in vivo*. EMBO J 10:2559–2567
10. Campanero MR, Armstrong MI, Flemington EK (2000) CpG methylation as a mechanism for the regulation of E2F activity. Proc Natl Acad Sci USA 97:6481–6486
11. Eden S, Hashimshony T, Keshet I, Cedar H, Thorne AW (1998) DNA methylation models histone acetylation. Nature 394:842
12. Bird AP, Wolffe AP (1999) Methylation-induced repression-belts, braces, and chromatin. Cell 99:451–454
13. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410:116–120
14. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 410:120–124
15. Tamaru H, Selker EU (2001) A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. Nature 414:277–283
16. Benvenuto G, Carpentieri ML, Salvatore P, Cindolo L, Bruni CB, Chiariotti L (1996) Cell-specific transcriptional regulation and reactivation of galectin-1 gene expression are controlled by DNA methylation of the promoter region. Mol Cell Biol 16:2736–2743
17. Hodge DR, Xiao W, Clausen PA, Heidecker G, Szyf M, Farrar WL (2001) Interleukin-6 regulation of the human DNA methyltransferase (HDNMT) gene in human erythroleukemia cells. J Biol Chem 276:39508–39511
18. Paroush Z, Keshet I, Yisraeli J, Cedar H (1990) Dynamics of demethylation and activation of the alpha-actin gene in myoblasts. Cell 63:1229–1237
19. Lubbert M, Brugger W, Mertelsmann R, Kanz L (1996) Developmental regulation of myeloid gene expression and demethylation during *ex vivo* culture of peripheral blood progenitor cells. Blood 87:447–455
20. Takei S, Fernandez D, Redford A, Toyoda H (1996) Methylation status of 5′-regulatory region of tumor necrosis factor alpha gene correlates with differentiation stages of monocytes. Biochem Biophys Res Commun 220:606–612
21. Avni O, Rao A (2000) T cell differentiation: a mechanistic view. Curr Opin Immunol 12:654–659
22. Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M, Fujita N, Nakao M, Taga T (2001) DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. Dev Cell 1:749–758
23. Cervoni N, Szyf M (2001) Demethylase activity is directed by histone acetylation. J Biol Chem 276:40778–40787
24. Miyata Y, Towatari M, Maeda T, Ozawa Y, Saito H (2001) Histone acetylation induced by granulocyte colony-stimulating factor in a map kinase-dependent manner. Biochem Biophys Res Commun 283:655–660

25. Muller C, Leutz A (2001) Chromatin remodeling in development and differentiation. Curr Opin Genet Dev 11:167–174
26. Sartorelli V, Puri PL, Hamamori Y, Ogryzko V, Chung G, Nakatani Y, Wang JY, Kedes L (1999) Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. Mol Cell 4:725–734
27. Huang S, Qiu Y, Shi Y, Xu Z, Brandt SJ (2000) P/CAF-mediated acetylation regulates the function of the basic helix-loop-helix transcription factor TAL1/SCL. EMBO J 19:6792–6803
28. Robertson KD (2001) DNA methylation, methyltransferases, and cancer. Oncogene 20:3139–3155
29. Rountree MR, Bachman KE, Herman JG, Baylin SB (2001) DNA methylation, chromatin inheritance, and cancer. Oncogene 20:3156–3165
30. Conway KE, McConnell BB, Bowring CE, Donald CD, Warren ST, Vertino PM (2000) TMS1, a novel proapoptotic caspase recruitment domain protein, is a target of methylation-induced gene silencing in human breast cancers. Cancer Res 60:6236–6242
31. Cairns BR (2001) Emerging roles for chromatin remodeling in cancer biology. Trends Cell Biol 11:S15–S21
32. Wolffe AP (2001) Chromatin remodeling: why it is important in cancer. Oncogene 20:2988–2990
33. Ginsburg H, Sachs L (1963) Formation of pure suspensions of mast cells in tissue culture by differentiation of lymphoid cells from the mouse thymus. J Natl Cancer Inst 31:1–40
34. Pluznik DH, Sachs L (1965) The cloning of normal mast cells in tissue culture. J Cell Comp Physiol 66:319–324
35. Ichikawa Y, Pluznik DH, Sachs L (1966) *In vitro* control of the development of macrophage and granulocyte colonies. Proc Natl Acad Sci USA 56:488–495
36. Pluznik DH, Sachs L (1966) The induction of clones of normal mast cells by a substance from conditioned medium. Exp Cell Res 43:553–563
37. Sachs L (1987) The molecular control of blood cell development. Science 238:1374–1379
38. Ichikawa Y, Pluznik DH, Sachs L (1967) Feedback inhibition of the development of macrophage and granulocyte colonies I Inhibition by macrophages. Proc Natl Acad Sci USA 58:1480–1486
39. Landau T, Sachs L (1971) Characterization of the inducer required for the development of macrophage and granulocyte colonies. Proc Natl Acad Sci USA 68:2540–2544
40. Metcalf D (1985) The granulocyte-macrophage colony-stimulating factors. Science 229:16–22
41. Ihle JN, Keller J, Henderson L, Klein F, Palaszinski E (1982) Procedures for the purification of interleukin-3 to homogeneity. J Immunol 129:2431–2436
42. Witte ON (1990) Steel locus defines new multipotent growth factor. Cell 63:5–6
43. Lyman SD, James L, Vanden Bos T, de Vries P, Brasel K, Gliniak B, Hollingsworth LT, Picha KS, McKenna HJ, Splett RR (1993) Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell 75:1157–1167
44. Kaushansky K (1995) Thrombopoietin: the primary regulator of platelet production. Blood 86:419–431
45. Eglitis MA, Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci USA 94:4080–4085
46. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279:1528–1530
47. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. Nat Med 6:1229–1234
48. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105:369–377
49. Shabo Y, Lotem J, Rubinstein M, Revel M, Clark SC, Wolf SF, Kamen R, Sachs L (1988) The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. Blood 72:2070–2073
50. Gearing DP, Gough NM, King JA, Hilton DJ, Nicola NA, Simpson RJ, Nice EC, Kelso A, Metcalf D (1987) Molecular cloning and expression of cDNA encoding a murine myeloid leukemia inhibitory factor (LIF). EMBO J 6:3995–4002
51. Lowe DG, Nunes W, Bombara M, McCabe S, Ranges GE, Henzel W, Tomida M, Yamamoto-Yamaguchi Y, Hozumi M, Goeddel DV (1989) Genomic cloning and heterologous expression of human differentiation-stimulating factor. DNA 8:351–359
52. Lotem J, Sachs L (1989) Indirect induction of differentiation of normal and leukemic myeloid cells by recombinant interleukin-1. Leuk Res 13:13–21
53. Lotem J, Sachs L (1990) Selective regulation of the activity of different hematopoietic regulatory proteins by transforming growth factor beta 1 in normal and leukemic myeloid cells. Blood 76:1315–1322
54. Taga T, Kishimoto T (1997) Gp130 and the interleukin-6 family of cytokines. Annu Rev Immunol 15:797–819
55. Lotem J, Sachs L (1982) Mechanisms that uncouple growth and differentiation in myeloid leukemia cells: restoration of requirement for normal growth-inducing protein without restoring induction of differentiation-inducing protein. Proc Natl Acad Sci USA 79:4347–4351
56. Sachs L, Lotem J (1994) The network of hematopoietic cytokines. Proc Soc Exp Biol Med 206:170–175
57. Sachs L (1990) The control of growth and differentiation in normal and leukemic blood cells. Cancer 65:2196–2206
58. Lotem J, Sachs L (2002) Cytokine control of developmental programs in normal hematopoiesis and leukemia. Oncogene 3284–3294
59. Fibach E, Landau T, Sachs L (1972) Normal differentiation of myeloid leukaemic cells induced by a differentiation-inducing protein. Nat New Biol 237:276–278
60. Olsson I, Arnljots K, Gullberq U, Lantz M, Peetre C, Richter J (1988) Myeloid cell differentiation: the differentiation inducing factors of myeloid leukemia cells. Leukemia 2:16S–23S
61. Fibach E, Hayashi M, Sachs L (1973) Control of normal differentiation of myeloid leukemic cells to macrophages and granulocytes. Proc Natl Acad Sci USA 70:343–346
62. Lotem J, Sachs L (1974) Different blocks in the differentiation of myeloid leukemic cells. Proc Natl Acad Sci USA 71:3507–3511
63. Lotem J, Sachs L (1978) *In vivo* induction of normal differentiation in myeloid leukemia cells. Proc Natl Acad Sci USA 75:3781–3785
64. Lotem J, Sachs L (1988) *In vivo* control of differentiation of myeloid leukemic cells by recombinant granulocyte-macrophage colony-stimulating factor and interleukin-3. Blood 71: 375–382
65. Gootwine E, Webb CG, Sachs L (1982) Participation of myeloid leukaemic cells injected into embryos in haematopoietic differentiation in adult mice. Nature 299:63–65
66. Webb CG, Gootwine E, Sachs L (1984) Developmental potential of myeloid leukemia cells injected into midgestation embryos. Dev Biol 101:221–224

67. Fearon ER, Burke PJ, Schiffer CA, Zehnbauer BA, Vogelstein B (1986) Differentiation of leukemia cells to polymorphonuclear leukocytes in patients with acute nonlymphocytic leukemia. N Engl J Med 315:15–24
68. Lotem J, Sachs L (1981) *In vivo* inhibition of the development of myeloid leukemia by injection of macrophage- and granulocyte-inducing protein. Int J Cancer 28:375–386
69. Lotem J, Sachs L (1984) Control of *in vivo* differentiation of myeloid leukemic cells. IV. Inhibition of leukemia development by myeloid differentiation-inducing protein. Int J Cancer 33:147–154
70. Sachs L (1978) Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukemia. Nature 274:535–539
71. Sachs L (1987) The Wellcome Foundation lecture, 1986. The molecular regulators of normal and leukaemic blood cells. Proc R Soc London B Biol Sci 231:289–312
72. Sachs L (1987) Cell differentiation and bypassing of genetic defects in the suppression of malignancy. Cancer Res 47:1981–1986
73. Marks PA, Rifkind RA (1978) Erythroleukemic differentiation. Annu Rev Biochem 47:419–448
74. Sachs L (1982) Normal developmental programmes in myeloid leukemia: regulatory proteins in the control of growth and differentiation. Cancer Surv 1:321–342
75. Deroo BJ, Archer TK (2001) Glucocorticoid receptor-mediated chromatin remodeling *in vivo*. Oncogene 20:3039–3046
76. Degos L, Wang ZY (2001) All *trans*-retinoic acid in acute promyelocytic leukemia. Oncogene 20:7140–7145
77. Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Lo Coco F, Kouzarides T, Nervi C, Minucci S, Pelicci PG (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 295:1079–1082
78. Zhu J, Lallemand-Breitenbach V, de The H (2001) Pathways of retinoic acid- or arsenic trioxide-induced PML/RARα catabolism, role of oncogene degradation in disease remission. Oncogene 20:7257–7265
79. Hong SH, Yang Z, Privalsky ML (2001) Arsenic trioxide is a potent inhibitor of the interaction of SMRT corepressor with its transcription factor partners, including the PML–retinoic acid receptor alpha oncoprotein found in human acute promyelocytic leukemia. Mol Cell Biol 21:7172–7182
80. Zhang TD, Chen GQ, Wang ZG, Wang ZY, Chen SJ, Chen Z (2001) Arsenic trioxide, a therapeutic agent for APL. Oncogene 20:7146–7153
81. Marks PA, Rifkind RA, Richon VM, Breslow RTM, Kelly WK (2001) Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 1:194–202
82. He LZ, Tolentino T, Grayson P, Zhong S, Warrell RPJ, Rifkind RA, Marks PA, Richon VM, Pandolfi PP (2001) Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. J Clin Invest 108:1321–1330
83. Wang J, Saunthararajah Y, Redner RL, Liu JM (1999) Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1–ETO leukemia cells. Cancer Res 59:2766–2769
84. Dore BT, Chomienne C, Momparler RL (1998) Effect of 5-aza-2′-deoxycytidine and Vitamin D3 analogs on growth and differentiation of human myeloid leukemic cells. Cancer Chemother Pharmacol 41:275–280
85. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat Genet 21:103–107
86. Cohen L, Sachs L (1981) Constitutive gene expression in myeloid leukemia and cell competence for induction of differentiation by the steroid dexamethasone. Proc Natl Acad Sci USA 78:353–357
87. Leszczyniecka M, Roberts T, Dent P, Grant S, Fisher PB (2001) Differentiation therapy of human cancer: basic science and clinical applications. Pharmacol Ther 90:105–156
88. Guzhova I, Hultquist A, Cetinkaya C, Nilsson K, Pahlman S, Larsson LG (2001) Interferon-gamma cooperates with retinoic acid and phorbol ester to induce differentiation and growth inhibition of human neuroblastoma cells. Int J Cancer 94:97–108
89. Deeble PD, Murphy DJ, Parsons SJ, Cox ME (2001) Interleukin-6- and cyclic AMP-mediated signaling potentiates neuroendocrine differentiation of LNCaP prostate tumor cells. Mol Cell Biol 21:8471–8482
90. Chen A, Davis BH, Bissonnette M, Scaglione-Sewell B, Brasitus TA (1999) 1,25-Dihydroxy Vitamin D3 stimulates activator protein-1-dependent caco-2 cell differentiation. J Biol Chem 274:35505–35513
91. McBain JA, Pettit GR, Mueller GC (1988) Bryostatin 1 antagonizes the terminal differentiating action of 12-*O*-tetradecanoylphorbol-13-acetate in a human colon cancer cell. Carcinogenesis 9:123–129
92. Nojiri H, Manya H, Isono H, Yamana H, Nojima S (1999) Induction of terminal differentiation and apoptosis in human colonic carcinoma cells by brefeldin A, a drug affecting ganglioside biosynthesis. FEBS Lett 453:140–144
93. Preis PN, Saya H, Nadasdi L, Hochhaus G, Levin V, Sadee W (1988) Neuronal cell differentiation of human neuroblastoma cells by retinoic acid plus herbimycin A. Cancer Res 48:6530–6534
94. Huang H, Reed CP, Zhang JS, Shridhar V, Wang L, Smith DI (1999) Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. Cancer Res 59:2981–2988
95. Munster PN, Troso-Sandoval T, Rosen N, Rifkind R, Marks PA, Richon VM (2001) The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces differentiation of human breast cancer cells. Cancer Res 61:8492–8497
96. Altucci L, Gronemeyer H (2001) The promise of retinoids to fight against cancer. Nat Rev Cancer 1:181–193