

Publications de l'E 2010 (Inserm) (2002-2011)

2011

HAUER J., MULLIGHAN C., MORILLON E., WANG G., BRUNEAU J., BROUSSE N., LELORC'H M., ROMANA S., BOUDIL A., TIEDAU D., KRACKER S., BUSHMANN F.D., BORKHARDT A., FISCHER A., HACEIN-BEY-ABINA S., CAVAZZANA-CALVO M.

Loss of p19Arf in a Rag1^{-/-} B-cell precursor population initiates acute B-lymphoblastic leukemia.

Blood, 118 (3), 544-553, 2011

(Services cités : Anatomie Pathologique, CIC-BT 502, E 0210, Immunologie-Hématologie Pédiatriques, U1020, U768)

In human B-acute lymphoblastic leukemia (B-ALL), RAG1-induced genomic alterations are important for disease progression. However, given that biallelic loss of the RAG1 locus is observed in a subset of cases, RAG1's role in the development of B-ALL remains unclear. We chose a p19Arf^(-/-)Rag1^(-/-) mouse model to confirm the previously published results concerning the contribution of CDKN2A (p19ARF /INK4a) and RAG1 copy number alterations in precursor B cells to the initiation and/or progression to B-acute lymphoblastic leukemia (B-ALL). In this murine model, we identified a new, Rag1-independent leukemia-initiating mechanism originating from a Sca1(+)CD19(+) precursor cell population and showed that Notch1 expression accelerates the cells' self-renewal capacity in vitro. In human RAG1-deficient BM, a similar CD34(+)CD19(+) population expressed p19ARF. These findings suggest that combined loss of p19Arf and Rag1 results in B-cell precursor leukemia in mice and may contribute to the progression of precursor B-ALL in humans.

2010

CHAPIRO E., LEPORRIER N., RADFORD-WEISS I., BASTARD C., MOSSAFA H., LEROUX D., TIGAUD I., de BRAEKELEER M., TERRE C., BRIZARD F., CALLET-BAUCHU E., STRUSKI S., VERONESE L., FERT-FERRER S., TAVIAUX S., LESTY C., DAVI F., MERLE-BERAL H., BERNARD O.A., SUTTON L., RAYNAUD S.D., NGUYEN-KHAC F.

Gain of the short arm of chromosome 2 (2p) is a frequent recurring chromosome aberration in untreated chronic lymphocytic leukemia (CLL) at advanced stages.

Leuk. Res., 34 (1), 63-68, 2010

(Services cités : E 0210, Histo-Embryologie - Cytogénétique)

Using array-based CGH, we identified 2p gain in 22/78 (28%) untreated Binet stages B/C CLL, which was the second most frequent copy number change after 13q deletion. It never occurred as a sole abnormality and was associated with other changes (6q deletion; 1p gain). The region of 2p gain frequently included two oncogenes, REL and MYCN. All patients with gain of REL were unmutated for IGHV (p=0.03). Gain of MYCN was associated with increased mRNA expression (p=0.005), suggesting a pathogenic role for MYCN. Gain of 2p appears to be a marker of progression and may contribute to the poor prognosis.

COURONNE L., LIPPERT E., ANDRIEUX J., KOSMIDER O., RADFORD-WEISS I., PENTHER D., DASTUGUE N., MUGNERET F., LAFAGE M., GACHARD N., NADAL N., BERNARD O.A., NGUYEN-KHAC F.

Analyses of TET2 mutations in post-myeloproliferative neoplasm acute myeloid leukemias.

Leukemia, 24 (1), 201-203, 2010

(Services cités : E 0210, Histo-Embryologie - Cytogénétique)

FERLICOT S., VERNOCHET A., ROMANA S., ORTIN-SERRANO M., LETIERCE A., BREGERIE O., DURRBACH A., GUETTIER C.

Microchimerism in renal allografts: clinicopathological associations according to the type of chimeric cells.

Histopathology, 56 (2), 188-197, 2010

(Services cités : E 0210)

Microchimerism in renal allografts: clinicopathological associations according to the type of chimeric cells Aims: Recent studies have highlighted the presence of microchimerism in various solid allografts. The biological significance of these chimeric cells is controversial. They may be beneficial, leading to better tolerance of grafts or participating in tissue repair or, in contrast, deleterious if involved in chronic lesions. The aim was to assess the frequency and cellular nature of microchimerism in female renal grafts of male recipients by combined fluorescence in situ hybridization (FISH) for Y chromosome and immunohistochemistry and to investigate associations between intragraft microchimerism and histological lesions or allograft outcome. Methods and results: We screened 33 renal biopsy specimens, including 11 with acute T-cell-

mediated rejection and nine with transplant glomerulopathy, from 22 male recipients transplanted with female kidneys by FISH and immunohistochemistry with antibodies against smooth muscle actin (mesangial cells), CD31 (endothelial cells), KL1 (epithelial cells), CD45 (leucocyte common antigen) and glomerular epithelial protein 1 (podocytes). Tubular microchimerism was detected in 71% of the patients with a mean percentage of chimeric epithelial cells of 1.4%. Glomerular microchimerism involving podocytes, mesangial and endothelial cells was present with a mean number of chimeric cells per glomerular section of, respectively, 0.6, 2.66 and 3.53. There was an association between endothelial microchimerism and a previous episode of acute T-cell-mediated rejection. Conclusions: In conclusion, microchimerism in renal grafts occurs frequently, but at a low level and affects tubular cells and all glomerular cell compartments in human renal allografts.

NGUYEN-KHAC F., BARIN C., CHAPIRO E., MACINTYRE E.A., ROMANA S., BERNARD O.A.

Cyclin D3 deregulation by juxtaposition with IGH locus in a t(6;14)(p21;q32)-positive T-cell acute lymphoblastic leukemia.

Leuk. Res., 34 (1), e13-e14, 2010

(Services cités : E 0210, Laboratoire d'Hématologie)

NIBOUREL O., KOSMIDER O., CHEOK M., BOISSEL N., RENNEVILLE A., PHILIPPE N., DOMBRET H., DREYFUS F., QUESNEL B., GEFFROY S., QUENTIN S., ROCHE-LESTIENNE C., CAYUELA J.M., ROUMIER C., FENAUX P., VAINCHENKER W., BERNARD O.A., SOULIER J., FONTENAY M., PREUDHOMME C.

Incidence and prognostic value of TET2 alterations in de novo acute myeloid leukemia achieving complete remission.

Blood, 116 (7), 1132-1135, 2010

(Services cités : E 0210)

Mutations of the ten eleven translocation 2 gene (TET2) have recently been reported in myelodysplastic syndrome and myeloproliferative neoplasms. We analyzed the incidence and prognostic value of TET2 point mutations and other genomic alterations by direct sequencing and single nucleotide polymorphism microarray analysis in 111 de novo acute myeloid leukemia, who had all achieved complete remission (CR). Mutations were observed in 19 (17%) of the 111 patients compared with 10 (27%) of 36 patients who had failed to achieve CR ($P = .2$). In the 111 patients who had achieved CR, TET2 alterations were only significantly associated with NPM1 mutations but not with other pretreatment characteristics. TET2 gene status was not significantly correlated with disease-free survival and overall survival, both in the entire cohort and in patients with normal karyotype.

PETIT A., RAGU C., DELLA VALLE V., MOZZICONACCI M.J., LAFAGE-POCHITALOFF M., SOLER G., SCHLUTH C., RADFORD I., OTTOLENGHI C., BERNARD O.A., PENARD-LACRONIQUE V., ROMANA S.P.

NUP98-HMGB3: a novel oncogenic fusion.

Leukemia, 24 (3), 654-658, 2010

(Services cités : E 0210, Histo-Embryologie - Cytogénétique)

2009

ASNAFI V., BUZYN A., LE NOIR S., BALEYDIER F., SIMON A., BELDJORD K., REMAN O., WITZ F., FAGOT T., TAVERNIER E., TURLURE P., LEGUAY T., HUGUET F., VERNANT J.P., DANIEL F., BENE M.C., IFRAH N., THOMAS X., DOMBRET H., MACINTYRE E.

NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study.

Blood, 113 (17), 3918-3924, 2009

(Services cités : E 0210, Hématologie Adulte, Laboratoire d'Hématologie)

Many somatic genetic abnormalities have been identified in T-cell acute lymphoblastic leukemia (T-ALL) but each individual abnormality accounts for a small proportion of cases; therapeutic stratification consequently still relies on classical clinical markers. NOTCH1 and/or FBXW7 mutations both lead to activation of the NOTCH1 pathway and are among the most frequent mutations in T-ALL. We screened 141 adult diagnostic T-ALL samples from patients treated on either the Lymphoblastic Acute Leukemia in Adults (LALA)-94 (n = 87) or the GRAALL-2003 (n = 54) trials. In 88 cases (62%) there were demonstrated NOTCH1 mutations (42% heterodimerization [HD], 10% HD+proline glutamate serine threonine [PEST], 6% PEST, 2% juxtamembrane mutations, 2% transactivation domain [TAD]) and 34 cases (24%) had FBXW7 mutations (21 cases had both NOTCH1 and FBXW7 mutations); 40 cases (28%) were wild type for both. There was no significant correlation between NOTCH1 and/or FBXW7 mutations and clinico-biologic features. Median event-free survival (EFS) and overall survival (OS) were 36 versus 17 months (P =.01) and not reached versus 32 months (P =.004) in patients with NOTCH1 and/or FBXW7 mutations versus other patients, respectively. Multivariate analysis showed that the presence of NOTCH1/FBXW7 mutations was an independent good prognostic factor for EFS and OS (P =.02 and P =.01, respectively). These data demonstrate that NOTCH1 pathway activation by either NOTCH1 or FBXW7 mutation identifies a large group of patients with a favorable outcome that could justify individual therapeutic stratification for T-ALL.

BERNARD O.A., DELHOMMEAU F., FONTENAY M., VAINCHENKER W.

Mutations in TET2 in myeloid cancers.

M S-Méd. Sci., 25 (10), 785-788, 2009

(Services cités : E 0210)

BERNARD O.A., GILLILAND D.G., MERCHER T.

The OTT-MAL fusion oncogene: another Notch in megakaryoblastic leukemia.

M S-Méd. Sci., 25 (8-9), 676-678, 2009

(Services cités : E 0210)

BERNARD O.A., VAINCHENKER W.

Mutation in TET2 in Myeloid Cancers AUTHORS REPLY.

N. Engl. J. Med., 361 (11), 1117-1118, 2009

(Services cités : [E 0210](#))

CARBUCCIA N., MURATI A., TROUPLIN V., BRECQUEVILLE M., ADELAIDE J., REY J., VAINCHENKER W., BERNARD O.A., CHAFFANET M., VEY N., BIRNBAUM D., MOZZICONACCI M.J.

Mutations of ASXL1 gene in myeloproliferative neoplasms.

Leukemia, 23 (11), 2183-2186, 2009

(Services cités : [E 0210](#))

CORNEJO M.G., BOGGON T.J., MERCHER T.

JAK3: a two-faced player in hematological disorders.

Int. J. Biochem. Cell Biol., 41 (12), 2376-2379, 2009

(Services cités : [E 0210](#))

JAK3 is a non-receptor tyrosine kinase, predominantly expressed in hematopoietic cells and that has been implicated in the signal transduction of the common gamma chain subfamily of cytokine receptors. As a result, JAK3 plays an essential role in hematopoiesis during T cell development. JAK3 inactivating mutations result in immunodeficiency syndromes (SCID) in both humans and mice. Recent data indicate that abnormal activation of JAK3 due to activating mutations is also found in human hematological malignancies, including acute megakaryoblastic leukemia (AMKL) and cutaneous T cell lymphoma (CTCL). After a brief summary of the JAK3 structure and function, we will review the evidence on the emerging role of JAK3 activation in hematological malignancies that warrant further studies to test the relevance of specific inhibition of JAK3 as a therapeutic approach to these challenging clinical entities.

CORNEJO M.G., KHARAS M.G., WERNECK M.B., LE BRAS S., MOORE S.A., BALL B., BEYLOT-BARRY M., RODIG S.J., ASTER J.C., LEE B.H., CANTOR H., MERLIO J.P., GILLILAND D.G., MERCHER T.

Constitutive JAK3 activation induces lymphoproliferative syndromes in murine bone marrow transplantation models.

Blood, 113 (12), 2746-2754, 2009

(Services cités : [E 0210](#))

The tyrosine kinase JAK3 plays a well-established role during normal lymphocyte development and is constitutively phosphorylated in several lymphoid malignancies. However, its contribution to lymphomagenesis remains elusive. In this study, we used the newly identified activating JAK3A572V mutation to elucidate the effect of constitutive JAK3 signaling on murine lymphopoiesis. In a bone marrow transplantation model, JAK3A572V induces an aggressive, fatal, and transplantable lymphoproliferative disorder characterized by the expansion of CD8(+)TCRalpha(+)CD44(+)CD122(+)Ly-6C(+) T cells that closely resemble an effector/memory T-cell subtype. Compared with wild-type counterparts, these cells show increased proliferative capacities in response to polyclonal stimulation, enhanced survival rates with elevated expression of Bcl-2, and increased production of interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF-alpha), correlating with enhanced cytotoxic abilities against

allogeneic target cells. Of interest, the JAK3A572V disease is epidermotropic and produces intraepidermal microabscesses. Taken together, these clinical features are reminiscent of those observed in an uncommon but aggressive subset of CD8(+) human cutaneous T-cell lymphomas (CTCLs). However, we also observed a CD4(+) CTCL-like phenotype when cells are transplanted in an MHC-I-deficient background. These data demonstrate that constitutive JAK3 activation disrupts T-cell homeostasis and induces lymphoproliferative diseases in mice.

DELHOMMEAU F., DUPONT S., DELLA-VALLE V., JAMES C., TRANNOY S., MASSE A., KOSMIDER O., LE COUEDIC J.P., ROBERT F., ALBERDI A., LECLUSE Y., PLO I., DREYFUS F.J., MARZAC C., CASADEVALL N., LACOMBE C., ROMANA S.P., DESSEN P., SOULIER J., VIGUIE F., FONTENAY M., VAINCHENKER W., BERNARD O.A.

Mutation in TET2 in myeloid cancers.

N. Engl. J. Med., 360 (22), 2289-2301, 2009

(Services cités : E 0210)

BACKGROUND: The myelodysplastic syndromes and myeloproliferative disorders are associated with deregulated production of myeloid cells. The mechanisms underlying these disorders are not well defined. **METHODS:** We conducted a combination of molecular, cytogenetic, comparative-genomic-hybridization, and single-nucleotide-polymorphism analyses to identify a candidate tumor-suppressor gene common to patients with myelodysplastic syndromes, myeloproliferative disorders, and acute myeloid leukemia (AML). The coding sequence of this gene, TET2, was determined in 320 patients. We analyzed the consequences of deletions or mutations in TET2 with the use of in vitro clonal assays and transplantation of human tumor cells into mice. **RESULTS:** We initially identified deletions or mutations in TET2 in three patients with myelodysplastic syndromes, in three of five patients with myeloproliferative disorders, in two patients with primary AML, and in one patient with secondary AML. We selected the six patients with myelodysplastic syndromes or AML because they carried acquired rearrangements on chromosome 4q24; we selected the five patients with myeloproliferative disorders because they carried a dominant clone in hematopoietic progenitor cells that was positive for the V617F mutation in the Janus kinase 2 (JAK2) gene. TET2 defects were observed in 15 of 81 patients with myelodysplastic syndromes (19%), in 24 of 198 patients with myeloproliferative disorders (12%) (with or without the JAK2 V617F mutation), in 5 of 21 patients with secondary AML (24%), and in 2 of 9 patients with chronic myelomonocytic leukemia (22%). TET2 defects were present in hematopoietic stem cells and preceded the JAK2 V617F mutation in the five samples from patients with myeloproliferative disorders that we analyzed. **CONCLUSIONS:** Somatic mutations in TET2 occur in about 15% of patients with various myeloid cancers.

GARNACHE-OTTOU F., FEUILLARD J., FERRAND C., BIICHLER S., TRIMOREAU F., SEILLES E., SALAUN V., GARAND R., LEPELLEY P., MAYNADIE M., KUHLEIN E., DECONINCK E., DALIPHARD S., CHAPEROT L., BESEGGIO L., FOISSEAUD V., MACINTYRE E., BENE M.C., SAAS P., JACOB M.C.

Extended diagnostic criteria for plasmacytoid dendritic cell leukaemia.

Br. J. Haematol., 145 (5), 624-636, 2009

(Services cités : E 0210, Laboratoire d'Hématologie)

The diagnosis of plasmacytoid dendritic cell leukaemia (pDCL) is based on the immunophenotypic profile: CD4(+) CD56(+) lineage(neg) CD45RA(+)/RO(neg) CD11c(neg) CD116(low) CD123(+) CD34(neg) CD36(+) HLA-DR(+). Several studies have reported pDCL cases that do not express this exact profile or expressing some lineage antigens that could thus be misdiagnosed. This study aimed to validate pDCL-specific markers for diagnosis by flow-cytometry or quantitative reverse transcription polymerase chain reaction on bone marrow samples. Expression of markers previously found in normal pDC was analysed in 16 pDCL, four pDCL presenting an atypical phenotype (apDCL) and 113 non-pDC - lymphoid or myeloid - acute leukaemia. CD123 was expressed at significantly higher levels in pDCL and apDCL. BDCA-2 was expressed on 12/16 pDCL and on 2/4 apDCL, but was never detected in the 113 non-pDC acute leukaemia cases. BDCA-4 expression was found on 13/16 pDCL, but also in 12% of non-pDC acute leukaemia. High levels of LILRA4 and TCL1A transcripts distinguished pDCL and apDCL from all other acute leukaemia (except B-cell acute lymphoblastic leukaemia for TCL1A). We thus propose a diagnosis strategy, scoring first the CD4(+) CD56(+/-) MPO(neg) cCD3(neg) cCD79a(neg) CD11c(neg) profile and then the CD123(high), BDCA-2 and BDCA-4 expression. Atypical pDCL can be also identified this way and non-pDC acute leukaemia excluded: this scoring strategy is useful for diagnosing pDCL and apDCL.

GILLES L., BLUTEAU D., BOUKOUR S., CHANG Y., ZHANG Y., ROBERT T., DESSEN P., DEBILI N., BERNARD O.A., VAINCHENKER W., RASLOVA H.

MAL/SRF complex is involved in platelet formation and megakaryocyte migration by regulating MYL9 (MLC2) and MMP9.

Blood, *114* (19), 4221-4232, 2009

(Services cités : [E 0210](#))

Megakaryoblastic leukemia 1 (MAL) is a transcriptional coactivator of serum response factor (SRF). In acute megakaryoblastic leukemia, the MAL gene is translocated and fused with the gene encoding one twenty-two (OTT). Herein, we show that MAL expression increases during the late differentiation steps of neonate and adult human megakaryopoiesis and localized into the nucleus after Rho GTPase activation by adhesion on collagen I or convulxin. MAL knockdown in megakaryocyte progenitors reduced the percentage of cells forming filopodia, lamellipodia, and stress fibers after adhesion on the same substrates, and reduced proplatelet formation. MAL repression led to dysmorphic megakaryocytes with disorganized demarcation membranes and alpha granules heterogeneously scattered in the cytoplasm. Gene expression profiling revealed a marked decrease in metalloproteinase 9 (MMP-9) and MYL9 expression after MAL inhibition. Luciferase assays in HEK293T cells and chromatin immunoprecipitation in primary megakaryocytes showed that the MAL/SRF complex directly regulates MYL9 and MMP9 in vitro. Megakaryocyte migration in response to stromal cell-derived factor 1, through Matrigel was considerably decreased after MAL knockdown, implicating MMP9 in migration. Finally, the use of a shRNA to decrease MYL9 expression showed that MYL9 was involved in proplatelet formation. MAL/SRF complex is thus involved in platelet formation and megakaryocyte migration by regulating MYL9 and MMP9.

KOSMIDER O., GELSI-BOYER V., CIUDAD M., RACOEUR C., JOOSTE V., VEY N., QUESNEL B., FENAUX P., BASTIE J.N., BEYNE-RAUZY O., STAMATOULAS A., DREYFUS F., IFRAH N., de BOTTON S., VAINCHENKER W., BERNARD O.A.,

BIRNBAUM D., FONTENAY M., SOLARY E.

TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia.

Haematologica, 94 (12), 1676-1681, 2009

(Services cités : E 0210)

BACKGROUND: Acquired somatic deletions and loss-of-function mutations in one or several codons of the TET2 (Ten-Eleven Translocation-2) gene were recently identified in hematopoietic cells from patients with myeloid malignancies, including myeloproliferative disorders and myelodys-plastic syndromes. The present study was designed to determine the prevalence of TET2 gene alterations in chronic myelomonocytic leukemias. **DESIGN AND METHODS:** Blood and bone marrow cells were collected from 88 patients with chronic phase chronic myelomonocytic leukemia and from 14 with acute transformation of a previously identified disease. Polymerase chain reaction analysis and direct sequencing were used to sequence exons 3 to 11 of the TET2 gene. Annotated single nucleotide polymorphisms were excluded. Survival curves were constructed by the Kaplan-Meier method. **RESULTS:** We detected TET2 mutations in 44 of 88 (50%) patients with chronic myelomonocytic leukemia, which suggests that TET2 gene mutations are especially frequent in this myeloid disease. A TET2 gene alteration was identified in 18 of the 43 patients studied at diagnosis and was associated with a trend to a lower overall survival rate; confining the analysis to the 29 patients with chronic myelomonocytic leukemia-1, according to the WHO classification, the difference in overall survival between patients with or without TET2 gene mutations became statistically significant. **CONCLUSIONS:** TET2 gene alterations are more frequent in chronic myelomonocytic leukemia than in other subgroups of hematopoietic diseases studied so far and could negatively affect the patients' outcome. The striking association between TET2 gene alterations and monocytosis, already observed in patients with systemic mastocytosis, could indicate a negative role of TET2 in the control of monocytic lineage determination.

MERCHER T., RAFFEL G.D., MOORE S.A., CORNEJO M.G., BAUDRY-BLUTEAU D., CAGNARD N., JESNECK J.L., PIKMAN Y., CULLEN D., WILLIAMS I.R., AKASHI K., SHIGEMATSU H., BOURQUIN J.P., GIOVANNINI M., VAINCHENKER W., LEVINE R.L., LEE B.H., BERNARD O.A., GILLILAND D.G.

The OTT-MAL fusion oncogene activates RBPJ-mediated transcription and induces acute megakaryoblastic leukemia in a knockin mouse model.

J. Clin. Invest., 119 (4), 852-864, 2009

(Services cités : E 0210, IRNEM)

Acute megakaryoblastic leukemia (AMKL) is a form of acute myeloid leukemia (AML) associated with a poor prognosis. The genetics and pathophysiology of AMKL are not well understood. We generated a knockin mouse model of the one twenty-two-megakaryocytic acute leukemia (OTT-MAL) fusion oncogene that results from the t(1;22)(p13;q13) translocation specifically associated with a subtype of pediatric AMKL. We report here that OTT-MAL expression deregulated transcriptional activity of the canonical Notch signaling pathway transcription factor recombination signal binding protein for immunoglobulin kappa J region (RBPJ) and caused abnormal fetal megakaryopoiesis. Furthermore, cooperation between OTT-MAL and an activating mutation of the thrombopoietin receptor myeloproliferative leukemia virus oncogene (MPL) efficiently induced a short-latency AMKL that recapitulated all the features of human AMKL, including megakaryoblast hyperproliferation and maturation block, thrombocytopenia, organomegaly, and extensive fibrosis. Our results establish that concomitant

activation of RBPJ (Notch signaling) and MPL (cytokine signaling) transforms cells of the megakaryocytic lineage and suggest that specific targeting of these pathways could be of therapeutic value for human AMKL.

MILPIED P., RENAND A., BRUNEAU J., MENDES DA CRUZ D.A., JACQUELIN S., ASNAFI V., RUBIO M.T., MACINTYRE E., LEPELLETIER Y., HERMINE O.

Neuropilin-1 is not a marker of human Foxp3(+) Treg.

Eur. J. Immunol., 39 (6), 1466-1471, 2009

(Services cités : Hématologie Adulte, UMR 8147, E 0210)

Treg are immune cells that play a critical role in the regulation of the immune response. Although the transcription factor Foxp3 is widely accepted as the standard marker of Treg, specific surface markers are needed to better characterize these cells and decipher their mechanisms of action. Neuropilin-1 (Nrp-1), a membrane protein primarily involved in the nervous system, was identified as a specific marker of murine Treg, but its expression has not been rigorously investigated in human Treg. Here we show that in contrast to murine Treg and regardless of their origins (blood, thymus, spleen, lymph node or tonsil), human Foxp3(+) Treg do not specifically express Nrp-1. However, a population of Foxp3(-) Nrp-1(+) T cells can be detected in human secondary lymphoid organs, and Nrp-1 expression is induced on peripheral blood T lymphocytes upon in vitro activation. We conclude that Nrp-1 cannot be used as a specific marker of human Treg, but might represent a novel activation marker of human T cells both in vitro and in vivo.

RAFFEL G.D., CHU G.C., JESNECK J.L., CULLEN D.E., BRONSON R.T., BERNARD O.A., GILLILAND D.G.

Ott1(Rbm15) is essential for placental vascular branching morphogenesis and embryonic development of the heart and spleen.

Mol. Cell. Biol., 29 (2), 333-341, 2009

(Services cités : E 0210)

The infant leukemia-associated gene, Ott1(Rbm15), has broad regulatory effects within murine hematopoiesis. However, germline Ott1 deletion results in fetal demise prior to E10.5, indicating additional developmental requirements for Ott1. The spen gene family, to which Ott1 belongs, has a transcriptional activation/repression domain and RNA recognition motifs, and in *Drosophila* has a significant role in the development of the head and thorax. Early Ott1-deficient embryos show growth retardation and incomplete closure of the notochord. Further analysis demonstrated placental defects in the spongiotrophoblast and syncytiotrophoblast layers, resulting in an arrest of vascular branching morphogenesis. Rescue of the placental defect using a conditional allele with a trophoblast-sparing cre transgene allowed embryos to form a normal placenta and survive gestation. This outcome showed that the process of vascular branching morphogenesis in Ott1-deficient animals was regulated by the trophoblast compartment rather than the fetal vasculature. Mice surviving to term manifested hyposplenia and abnormal cardiac development. Analysis of global gene expression of Ott1-deficient embryonic hearts showed enrichment of hypoxia-related genes and significant alteration of several candidate genes critical for cardiac development. Thus, Ott1-dependent pathways in addition to being implicated in leukemogenesis, may also be important in the pathogenesis of placental insufficiency and cardiac malformations.

RUSSELL L.J., CAPASSO M., VATER I., AKASAKA T., BERNARD O.A., CALASANZ M.J., CHANDRASEKARAN T., CHAPIRO E., GESK S., GRIFFITHS M., GUTTERY D.S., HAFERLACH C., HARDER L., HEIDENREICH O., IRVING J., KEARNEY L., NGUYEN-KHAC F., MACHADO L., MINTO L., MAJID A., MOORMAN A.V., MORRISON H., RAND V., STREFFORD J.C., SCHWAB C., TONNIES H., DYER M.J., SIEBERT R., HARRISON C.J.

Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B cell precursor acute lymphoblastic leukemia.

Blood, 114 (13), 2688-2698, 2009

(Services cités : [E 0210](#))

We report two novel, cryptic chromosomal abnormalities in precursor B-cell acute lymphoblastic leukemia (BCP-ALL): a translocation, either t(X;14)(p22;q32) or t(Y;14)(p11;q32), in 33 patients and an interstitial deletion, either del(X)(p22.33p22.33) or del(Y)(p11.32p11.32), in 64 patients, involving the pseudoautosomal region (PAR1) of the sex chromosomes. The incidence of these abnormalities was 5% in childhood ALL (0.8% with the translocation, 4.2% with the deletion). Patients with the translocation were older (median age 16 years), whilst the patients with the deletion were younger (median age 4 years). The two abnormalities result in deregulated expression of the cytokine receptor, cytokine receptor-like factor 2, CRLF2 (also known as thymic stromal-derived lymphopoietin receptor, TSLPR). Over-expression of CRLF2 was associated with activation of the JAK-STAT pathway in cell lines and transduced primary B-cell progenitors, sustaining their proliferation and indicating a causal role of CRLF2 over-expression in lymphoid transformation. In Down Syndrome (DS) ALL and two non DS BCP-ALL cell lines, CRLF2 deregulation was associated with mutations of the JAK2 pseudokinase domain suggesting oncogenic cooperation, as well as highlighting a link between non DS ALL and JAK2 mutations.

RUSSELL L.J., de CASTRO D.G., GRIFFITHS M., TELFORD N., BERNARD O., PANZER-GRUMAYER R., HEIDENREICH O., MOORMAN A.V., HARRISON C.J.

A novel translocation, t(14;19)(q32;p13), involving IGH@ and the cytokine receptor for erythropoietin.

Leukemia, 23 (3), 614-617, 2009

(Services cités : [E 0210](#))

TEFFERI A., PARDANANI A., LIM K.H., ABDEL-WAHAB O., LASHO T.L., PATEL J., GANGAT N., FINKE C.M., SCHWAGER S., MULLALLY A., LI C.Y., HANSON C.A., MESA R., BERNARD O., DELHOMMEAU F., VAINCHENKER W., GILLILAND D.G., LEVINE R.L.

TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis.

Leukemia, 23 (5), 905-911, 2009

(Services cités : [E 0210](#))

High-throughput DNA sequence analysis was used to screen for TET2 mutations in bone marrow-derived DNA from 239 patients with BCR-ABL-negative myeloproliferative neoplasms (MPNs). Thirty-two mutations (19 frameshift, 10 nonsense, 3 missense; mostly involving exons 4

and 12) were identified for an overall mutational frequency of approximately 13%. Specific diagnoses included polycythemia vera (PV; n=89), essential thrombocythemia (ET; n=57), primary myelofibrosis (PMF; n=60), post-PV MF (n=14), post-ET MF (n=7) and blast phase PV/ET/MF (n=12); the corresponding mutational frequencies were approximately 16, 5, 17, 14, 14 and 17% (P=0.50). Mutant TET2 was detected in approximately 17 and approximately 7% of JAK2V617F-positive and -negative cases, respectively (P=0.04). However, this apparent clustering of the two mutations was accounted for by an independent association between mutant TET2 and advanced age; mutational frequency was approximately 23% in patients > or =60 years old versus approximately 4% in younger patients (P<0.0001). The presence of mutant TET2 did not affect survival, leukemic transformation or thrombosis in either PV or PMF; a correlation with hemoglobin <10 g per 100 ml in PMF was noted (P=0.05). We conclude that TET2 mutations occur in both JAK2V617F-positive and -negative MPN, are more prevalent in older patients, display similar frequencies across MPN subcategories and disease stages, and hold limited prognostic relevance.

2008

BALEYDIER F., DECOUVELAERE A.V., BERGERON J., GAULARD P., CANIONI D., BERTRAND Y., LEPRETRE S., PETIT B., DOMBRET H., BELDJORD K., MOLINA T., ASNAFI V., MACINTYRE E.

T Cell Receptor Genotyping and HOXA/TLX1 Expression Define Three T Lymphoblastic Lymphoma Subsets which Might Affect Clinical Outcome.

Clin. Cancer Res., 14 (3), 692-700, 2008

(Services cités : E 0210, Laboratoire d'Hématologie, Anatomie Pathologique)

PURPOSE: T lymphoblastic lymphomas (T-LBL) are rare disorders of immature T cells which predominantly involve the mediastinum. Their oncogenic pathways and prognostic variables are not clear. **EXPERIMENTAL DESIGN:** We undertook a retrospective study of 41 cytoplasmic CD3+ T-LBL (nine cases aged <16 years) by assessing stage of maturation arrest based on T cell receptor (TCR) immunogenotyping, immunohistochemistry, and quantification of the oncogenes thought to be important in immature T cell malignancies. **RESULTS:** Application of a TCR-based immunogenetic classification allowed the identification of three subcategories: 11 immature IM0/D-LBL showed no TCR or only incomplete TCRD DJ rearrangement and corresponded to cytoplasmic CD3+ precursors of uncertain lineage. Sixteen mature TCRD(del)-LBL showed biallelic TCRD deletion and both TCRG and TCRB rearrangement, consistent with TCRalpha lineage restriction. Fourteen intermediate LBL (Int-LBL) showed complete TCRD VDJ and TCRG VJ rearrangement, with TCRB VDJ rearrangement in the majority. All Int-LBL expressed HOXA11/TLX1 or HOXA9 transcripts and a proportion of the latter were associated with CALM-AF10 or NUP214-ABL fusion transcripts. IM0/D-LBL were restricted to adults with extrathymic disease and bone marrow involvement, whereas Int-LBL and TCRD(del)-LBL were found in children and adults with predominantly thymic disease. In adults, the Int-LBL subgroup was associated with a significantly superior clinical outcome. This subgroup can be identified either by TCR immunogenotyping or HOXA9/TLX1 transcript quantification. **CONCLUSION:** Application of this molecular classification will allow the prospective evaluation of prognostic effects within pediatric and adult protocols.

BALLERINI P., LANDMAN-PARKER J., CAYUELA J.M., ASNAFI V., LABOPIN M., GANDEMER V., PEREL Y., MICHEL G., LEBLANC T., SCHMITT C., FASOLA S., HAGEMEJIER A., SIGAUX F., AUCLERC M.F., DOUAY L., LEVERGER G., BARUCHEL A.

Impact of genotype on survival of children with T-cell acute lymphoblastic leukemia treated according to the French protocol FRALLE-93: the effect of TLX3/HOX11L2 gene expression on outcome.

Haematologica, 93 (11), 1658-1665, 2008

(Services cités : E 0210, Laboratoire d'Hématologie)

BACKGROUND: The prognostic value of the ectopic activation of TLX3 gene expression, a major oncogenetic event associated with pediatric T-cell acute lymphoblastic leukemia, is controversial. Likewise, the frequency and the prognostic significance in pediatric T-cell acute lymphoblastic leukemia of the newly characterized NUP214-ABL1 fusion transcript is not yet clear. **DESIGN AND METHODS:** Two hundred children with T-cell acute lymphoblastic leukemia were treated in the French FRALLE-93 study from 1993 to 1999. The expression of TLX3, TLX1 and SILTAL1 genes was analyzed in samples from 92 patients by real-time quantitative reverse transcriptase polymerase chain reaction. Most of these samples were further

studied for NUP214-ABL1 and CALM-AF10 fusion transcripts. RESULTS: The median follow-up was 7.9 years. At 5 years the overall survival (+/- standard deviation, %) was 62 (+/-3%) and leukemia-free survival was 58 (+/-3%). Patients with T-cell acute lymphoblastic leukemia positive for TLX3 had a poorer survival compared to those with T-ALL negative for TLX3 (overall survival: 45+/-11% vs. 57+/-5%, p=0.049). In multivariate analysis, TLX3 expression was an independent adverse risk factor predicting relapse with a hazard ratio of 2.44 (p=0.017) and an overall survival with a hazard ratio of 3.7 (p=0.001). NUP214-ABL1 was expressed in 16.6% of patients with TLX3-positive T-ALL (3 of 18); all of the patients with this association died before completion of the treatment. SILTAL expression did not significantly affect the prognosis of patients with T-cell acute lymphoblastic leukemia. Only three of 92 patients expressed the TLX1 gene and all three are alive. CONCLUSIONS: TLX3 gene expression is an independent risk factor predicting poor survival in childhood T-cell acute lymphoblastic leukemia. When co-expressed with TLX3, NUP214-ABL1 transcripts may increase the risk of poor survival.

BERGER R., JONVEAUX P.

Cytogenetics 2008 and "Pathologie et Biologie".

Pathol. Biol., 56 (6), 343-344, 2008

(Services cités : [E 0210](#))

BERGER R., NGUYEN-KHAC F.

Chromosomal abnormalities and Waldenstrom macroglobulinemia.

Pathol. Biol., 56 (6), 400-406, 2008

(Services cités : [E 0210](#))

Waldenstrom macroglobulinemia (WM) is now defined as an uncommon lymphoplasmocytic proliferation associated with an immunoglobulin M peak. The associated chromosomal abnormalities are not specific to the disease, and changes in the diagnostic criteria and techniques used as well as low-level abnormal cell proliferation made their analysis difficult. A literature review however, shows that if specific abnormalities were not recognized until now, it is the frequency of some chromosomal abnormalities (for instance partial deletion of the long arm of chromosome 6 and trisomy 4) that distinguishes WM from other chronic malignant B-cell proliferations. The data collected in the present review show directions for future research which will benefit from use of more recent techniques such as fluorescent in situ hybridization, comparative genomic hybridization and expression microarrays.

BERNHEIM A., TOUJANI S., GUILLAUD-BATAILLE M., RICHON C., WAXIN H., DESSEN P., BERGER R.

Intragenic breakpoints localized by array CGH in a t(2;6) familial translocation.

Cytogenet. Genome Res., 119 (3-4), 185-190, 2008

(Services cités : [E 0210](#))

A 244K genome-wide array based comparative genomic hybridization study was carried out in a familial translocation t(2;6)(p25;p21) balanced in the mother and unbalanced in her daughter. In the past, this translocation has allowed us to localize the HLA multigene cluster to chromosome

6. With microarray technology, confirmation of the chromosome localization of the HLA system was easily obtained, showing that such approach may be applied to the breakpoint localizations of other familial structural changes when they are unbalanced. The disruption of genes at the translocation breakpoints that did not have any phenotypic consequences in the parent will allow the generation of a map of 'haplotolerant genes'. In addition, many genomic variants were detected with this technology, enlarging the possibility of analyzing their possible contribution to phenotypic diversity.

CHAPIRO E., RADFORD-WEISS I., BASTARD C., LUQUET I., LEFEBVRE C., CALLET-BAUCHU E., LEROUX D., TALMANT P., MOZZICONACCI M.J., MUGNERET F., STRUSKI S., RAYNAUD S., ANDRIEUX J., BARIN C., JOTTERAND M., MOSSAFA H., RAMOND S., TERRE C., LIPPERT E., BERGER F., FELMAN P., MERLE-BERAL H., BERNARD O.A., DAVI F., BERGER R., NGUYEN-KHAC F.

The most frequent t(14;19)(q32;q13)-positive B-cell malignancy corresponds to an aggressive subgroup of atypical chronic lymphocytic leukemia.

Leukemia, 22 (11), 2123-2127, 2008

(Services cités : [E 0210](#))

DESCOT A., REX-HAFFNER M., COURTOIS G., BLUTEAU D., MENSSEN A., MERCHER T., BERNARD O.A., TREISMAN R., POSERN G.

OTT-MAL is a deregulated activator of SRF-dependent gene expression.

Mol. Cell. Biol., 28 (20), 6171-6181, 2008

(Services cités : [E 0210](#))

The OTT-MAL/RBM15-MKL1 fusion protein is the result of the recurrent translocation t(1;22) in acute megakaryocytic leukaemia in infants. How it contributes to the malignancy is unknown. The 3' fusion partner, MAL/MKL1/MRTF-A, is a transcriptional coactivator of serum response factor (SRF). MAL plays a key role in regulated gene expression depending on Rho family GTPases and G-actin. Here we demonstrate that OTT-MAL is a constitutive activator of SRF and target gene expression. This requires the SRF binding motif and the MAL-derived transactivation domain. OTT-MAL localises to the nucleus and is not regulated by upstream signalling. OTT-MAL deregulation reflects its independence from control by G-actin, which fails to interact with OTT-MAL in co-immunoprecipitation experiments. Regulation cannot be restored by re-introduction of the entire MAL N-terminus into the fusion protein. OTT-MAL also caused a delayed induction of the MAL-independent, TCF-dependent target genes c-fos and egr-1, and the MAPK/Erk pathway. When tested in heterologous tissue culture systems, however, we observed considerable anti-proliferative effects of OTT-MAL. Our data suggest that the deregulated activation of MAL-dependent and -independent promoters results in tissue-specific functions of OTT-MAL.

HACEIN-BEY S., GARRIGUE A., WANG G.P., SOULIER J., LIM A., MORILLON E., CLAPPIER E., CACCAVELLI L., DELABESSE E., BELDJORD K., ASNAFI V., MACINTYRE E., DAL-CORTIVO L., RADFORD-WEISS I., BROUSSE N., SIGAUX F., MOSHOUS D., HAUER J., BORKHARDT A., BELOHRADSKY B.H., WINTERGERST

U., VELEZ M.C., LEIVA L., SORENSEN R., WULFFRAAT N., BLANCHE S., BUSHMAN F.D., FISCHER A., CAVAZZANA-CALVO M.

Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1.

J. Clin. Invest., 118 (9), 3132-3142, 2008

(Services cités : Anatomie Pathologique, Biothérapie, E 0210, Immuno-Hématologie-Rhumatologie Pédiatriques, Laboratoire d'Hématologie, U768, CIC-BT)

Previously, several individuals with X-linked SCID (SCID-X1) were treated by gene therapy to restore the missing IL-2 receptor gamma (IL2RG) gene to CD34(+) BM precursor cells using gammaretroviral vectors. While 9 of 10 patients were successfully treated, 4 of the 9 developed T cell leukemia 31-68 months after gene therapy. In 2 of these cases, blast cells contained activating vector insertions near the LIM domain-only 2 (LMO2) proto-oncogene. Here, we report data on the 2 most recent adverse events, which occurred in patients 7 and 10. In patient 10, blast cells contained an integrated vector near LMO2 and a second integrated vector near the proto-oncogene BMI1. In patient 7, blast cells contained an integrated vector near a third proto-oncogene, CCND2. Additional genetic abnormalities in the patients' blast cells included chromosomal translocations, gain-of-function mutations activating NOTCH1, and copy number changes, including deletion of tumor suppressor gene CDKN2A, 6q interstitial losses, and SIL-TAL1 rearrangement. These findings functionally specify a genetic network that controls growth in T cell progenitors. Chemotherapy led to sustained remission in 3 of the 4 cases of T cell leukemia, but failed in the fourth. Successful chemotherapy was associated with restoration of polyclonal transduced T cell populations. As a result, the treated patients continued to benefit from therapeutic gene transfer.

LORIAUX M.M., LEVINE R.L., TYNER J.W., FROHLING S., SCHOLL C., STOFFREGEN E.P., WERNIG G., ERICKSON H., EIDE C.A., BERGER R., BERNARD O.A., GRIFFIN J.D., STONE R.M., LEE B., MEYERSON M., HEINRICH M.C., DEININGER M.W., GILLILAND D.G., DRUKER B.J.

High-throughput sequence analysis of the tyrosine kinome in acute myeloid leukemia.

Blood, 111 (9), 4788-4796, 2008

(Services cités : E 0210)

To determine whether aberrantly activated tyrosine kinases other than FLT3 and c-KIT contribute to acute myeloid leukemia (AML) pathogenesis, we used high-throughput (HT) DNA sequence analysis to screen exons encoding the activation loop and juxtamembrane domains of 85 tyrosine kinase genes in 188 AML patients without FLT3 or c-KIT mutations. The screen identified 30 nonsynonymous sequence variations in 22 different kinases not previously reported in single-nucleotide polymorphism (SNP) databases. These included a novel FLT3 activating allele and a previously described activating mutation in MET (METT1010I). The majority of novel sequence variants were stably expressed in factor-dependent Ba/F3 cells. Apart from one FLT3 allele, none of the novel variants showed constitutive phosphorylation by immunoblot analysis and none transformed Ba/F3 cells to factor-independent growth. These findings indicate the majority of these alleles are not potent tyrosine kinase activators in this cellular context and that a significant proportion of nonsynonymous sequence variants identified in HT DNA sequencing screens may not have functional significance. Although some sequence variants may represent SNPs, these data are consistent with recent reports that a significant fraction of such sequence variants are "passenger" rather than "driver" alleles and underscore the importance of functional assessment of candidate disease alleles.

MALINGE S., RAGU C., DELLA VALLE V., PISANI D., CONSTANTINESCU S.N., PEREZ C., VILLEVAL J.L., REINHARDT D., LANDMAN-PARKER J., MICHAUX L., DASTUGUE N., BARUCHEL A., VAINCHENKER W., BOURQUIN J.P., PENARD-LACRONIQUE V., BERNARD O.A.

Activating mutations in human acute megakaryoblastic leukemia.

Blood, 112 (10), 4220-4226, 2008

(Services cités : E 0210)

Oncogenic activation of tyrosine kinase signaling pathway is recurrent in human leukemia. To gain insight into the oncogenic process leading to acute megakaryoblastic leukemia (AMKL) we performed sequence analyses of a subset of oncogenes known to be activated in human myeloid and myeloproliferative disorders. In a series of human AMKL samples from both Down Syndrome and non-Down Syndrome patients, mutations were identified within KIT, FLT3, JAK2, JAK3 and MPL genes with a higher frequency in DS than in non-DS patients. The novel mutations were analyzed using BaF3 cells, showing that JAK3 mutations were activating mutations. Finally, we report a novel constitutively active MPL mutant, MPLT487A, observed in a non-Down Syndrome childhood AMKL that induces a myeloproliferative disease in mouse bone marrow transplantation assay.

MERCHER T., CORNEJO M.G., SEARS C., KINDLER T., MOORE S.A., MAILLARD I., PEAR W.S., ASTER J.C., GILLILAND D.G.

Notch signaling specifies megakaryocyte development from hematopoietic stem cells.

Cell Stem Cell, 3 (3), 314-326, 2008

(Services cités : E 0210)

In the hematopoietic system, Notch signaling specifies T cell lineage fate, in part through negative regulation of B cell and myeloid lineage development. However, we unexpectedly observed the development of megakaryocytes when using heterotypic cocultures of hematopoietic stem cells with OP9 cells expressing Delta-like1, but not with parental OP9 cells. This effect was abrogated by inhibition of Notch signaling either with gamma-secretase inhibitors or by expression of the dominant-negative Mastermind-like1. The importance of Notch signaling for megakaryopoietic development in vivo was confirmed by using mutant alleles that either activate or inhibit Notch signaling. These findings indicate that Notch is a positive regulator of megakaryopoiesis and plays a more complex role in cell-fate decisions among myeloid progenitors than previously appreciated.

PETIT A., RADFORD-WEISS I., WAILL M.C., ROMANA S., BERGER R.

NUP98-NSD1 fusion by insertion in acute myeloblastic leukemia.

Cancer Genet. Cytogenet., 180 (1), 43-46, 2008

(Services cités : E 0210, Histo-Embryologie - Cytogénétique, IRNEM)

A case of NUP98-NSD1 gene fusion resulting from the insertion of a subtelomeric part of chromosome 11p15.4 within the subtelomeric part of 5q35 was detected in a child with acute myeloblastic leukemia. This new case illustrates the importance of using fluorescence in situ hybridization followed by reverse transcriptase-polymerase chain reaction techniques to detect abnormalities involving subtelomeric chromosomal regions.

SOLER G., RADFORD-WEISS I., BEN ABDELALI R., MAHLAOUI N., PONCEAU J.F., MACINTYRE E.A., VEKEMANS M., BERNARD O.A., ROMANA S.P.

Fusion of ZMIZ1 to ABL1 in a B-cell acute lymphoblastic leukaemia with a t(9;10)(q34;q22.3) translocation.

Leukemia, 22 (6), 1278-1280, 2008

(Services cités : E 0210, Laboratoire d'Hématologie, Immuno-Hématologie-Rhumatologie Pédiatriques)

SOLER G., RADFORD-WEISS I., MEYER C., MARSCHALEK R., BROUZES C., GHEZ D., ROMANA S., BERGER R.

MLL insertion with MLL-MLLT3 gene fusion in acute leukemia: case report and review of the literature.

Cancer Genet. Cytogenet., 183 (1), 53-59, 2008

(Services cités : E 0210, Hématologie Adulte, Histo-Embryologie - Cytogénétique, Laboratoire d'Hématologie)

A new chromosomal insertion involving the MLL gene was detected by fluorescence in situ hybridization in a patient with acute myeloblastic leukemia (AML) and a t(9;11)(p21;q13). Genomic polymerase chain reaction confirmed the MLL-MLLT3 gene fusion. A review of the literature on MLL insertions shows that the opposite orientation of the genes involved in the fusion plays a role in the genesis of the rearrangement in most of the cases reported.

SU X., DELLA-VALLE V., DELABESSE E., AZGUI Z., BERGER R., MERLE-BERAL H., BERNARD O.A., NGUYEN-KHAC F.

Transcriptional activation of the cardiac homeobox gene CSX1/NKX2-5 in a B-cell chronic lymphoproliferative disorder.

Haematologica, 93 (7), 1081-1085, 2008

(Services cités : E 0210)

Homeobox containing transcription factors are frequently deregulated in human hematologic malignant diseases either indirectly through an abnormality of an upstream factor, or directly through rearrangement of the gene itself. Study of T-cell acute lymphoblastic leukemia identified the related non-clustered homeobox transcription factors, TLX1 and TLX3, as frequently ectopically expressed as a result of chromosomal translocations. We report the deregulation of a non-clustered homeobox gene in a new type of t(5;14)(q35;q11) translocation in a mature peripheral B-cell leukemia. This translocation results in the ectopic expression of the CSX1/NKX2-5 gene on chromosome 5q35 due to its juxtaposition to the TCR delta gene on chromosome 14q11. Expression of the CSX1/NKX2-5 protein conferred enhanced replating potential to transduced murine bone marrow cells. Our study establishes that deregulation of homeobox encoding genes is not restricted to acute leukemic proliferations, but is also observed in chronic malignant diseases.

2007

BERGER R.

Three-way translocation involving band 6q21 in an acute lymphoblastic leukemia.

Cancer Genet. Cytogenet., 174 (1), 74-75, 2007

(Services cités : E 0210)

BERGER R., BERNARD O.A.

Interleukin-2 receptor beta chain locus rearrangement in a T-cell acute lymphoblastic leukemia.

Pathol. Biol., 55 (1), 56-58, 2007

(Services cités : E 0210)

A translocation t(1;22)(p13;q13) was detected in a child with T-cell acute lymphoblastic leukemia (T-ALL). FISH studies showed that the breakpoint was located in the 5' part of the interleukin-2 receptor beta chain (IL2RB) locus, but could only be located distal to 1p13.3 on the partner chromosome. This is the first case of the IL2RB locus rearrangement in T-ALL. The localization of the breakpoint suggests that the chromosomal translocation results in deregulation of IL2RB expression.

BERGER R.

Human cytogenetics. From 1956 to 2006.

Pathol. Biol., 55 (1), 1-12, 2007

(Services cités : E 0210)

The correct enumeration of human chromosomes, only established in 1956, has marked the starting point of the modern cytogenetics. The introduction of banding techniques, then of in situ hybridization techniques, and now of genomic microarray technology allowed a dramatic development of cytogenetics of which the main applications to basic and medical research are evoked in this review.

BERGER R., BERNARD O.A.

Jumping translocations.

Genes Chromosome Cancer, 46 (8), 717-723, 2007

(Services cités : E 0210)

Jumping translocations (JT) are uncommon constitutional or acquired chromosome rearrangements involving one donor and several recipient chromosomes. They occur in various pathologic conditions and the mechanism of their formation remains elusive. A review of the literature showed that the major localizations of the breakpoints of JTs in human samples are nonrandomly located in pericentromeric and telomeric regions of chromosomes. Interestingly, comparison of the localization of the chromosomal breakpoints and of presence of interstitial DNA repeats showed differences between constitutional and acquired JTs suggesting differences in the mechanisms for the genesis of JTs and their consequences. (c) 2007 Wiley-Liss, Inc.

BERGERON J., CLAPPIER E., RADFORD-WEISS I., BUZYN A., MILLIEN C., SOLER G., BALLERINI P., THOMAS X., SOULIER J., DOMBRET H., MACINTYRE E.A., ASNAFI V.

Prognostic and oncogenic relevance of TLX1/HOX11 expression level in T-ALLs.

Blood, 110 (7), 2324-2330, 2007

(Services cités : E 0210, Laboratoire d'Hématologie)

TLX1 is a homeodomain transcription factor generally associated with a favourable outcome in T-ALL. However, the molecular mechanisms of TLX1 deregulation remain unclear and various transcript levels in the absence of 10q24 abnormalities have been reported. A reproducible and accurate delineation of TLX1+ T-ALL will be necessary for proper therapeutic stratification. We have studied 264 unselected T-ALLs (171 adults and 93 children) and show that T-ALLs expressing high levels of TLX1 (n=35, 13%), defined as a RQ-PCR ratio of TLX1>1.00E+00 ABL, form a homogeneous oncogenic group, based on their uniform stage of maturation arrest and oncogenetic and transcriptional profiles. Furthermore, TLX1-high T-ALLs harbour molecular TLX1 locus abnormalities in the vast majority (31/33), a proportion largely underestimated by standard karyotypic screening. T-ALLs expressing TLX1 at lower levels (n=57, 22%) do not share these characteristics. Prognostic analysis within the adult LALA94 and GRAALL03 prospective protocols demonstrate a better event-free (p=0,035) and a marked trend for longer overall survival (p=0,059) for TLX1-high T-ALLs, while the expression of lower levels of TLX1 does not impact on prognosis. We propose that TLX1+ T-ALLs be defined as cases expressing TLX1/ABL ratios >1 and/or demonstrating TLX1 rearrangement. Therapeutic modification should be considered for those patients.

CAUWELIER B., CAVE H., GERVAIS C., LESSARD M., BARIN C., PEROT C., VAN DEN AKKER J., MUGNERET F., CHARRIN C., PAGES M.P., GREGOIRE M.J., JONVEAUX P., LAFAGE-POCHITALOFF M., MOZZICCONACCI M.J., TERRE C., LUQUET I., CORNILLET-LEFEBVRE P., LAURENCE B., PLESSIS G., LEFEBVRE C., LEROUX D., ANTOINE-POIREL H., GRAUX C., MAUVIEUX L., HEIMANN P., CHALAS C., CLAPPIER E., VERHASSELT B., BENOIT Y., MOERLOOSE B.D., POPPE B., VAN ROY N., KEERSMAECKER K.D., COOLS J., SIGAUX F., SOULIER J., HAGEMEIJER A., PAEPE A.D., DASTUGUE N., BERGER R., SPELEMAN F.

Clinical, cytogenetic and molecular characteristics of 14 T-ALL patients carrying the TCRbeta-HOXA rearrangement: a study of the Groupe Francophone de Cytogenetique Hematologique.

Leukemia, 21 (1), 121-128, 2007

(Services cités : E 0210)

Recently, we and others described a new chromosomal rearrangement, that is, inv(7)(p15q34) and t(7;7)(p15;q34) involving the T-cell receptor beta (TCRbeta) (7q34) and the HOXA gene locus (7p15) in 5% of T-cell acute lymphoblastic leukemia (T-ALL) patients leading to transcriptional activation of especially HOXA10. To further address the clinical, immunophenotypical and molecular genetic findings of this chromosomal aberration, we studied 330 additional T-ALLs. This revealed TCRbeta-HOXA rearrangements in five additional patients, which brings the total to 14 cases in 424 patients (3.3%). Real-time quantitative PCR analysis for HOXA10 gene expression was performed in 170 T-ALL patients and detected HOXA10 overexpression in 25.2% of cases including all the cases with a TCRbeta-HOXA rearrangement (8.2%). In contrast, expression of the short HOXA10 transcript, HOXA10b, was almost exclusively found in the TCRbeta-HOXA rearranged cases, suggesting a specific role for the HOXA10b short transcript in TCRbeta-HOXA-mediated oncogenesis. Other molecular and/or cytogenetic aberrations frequently found in subtypes of T-ALL (SIL-TAL1, CALM-AF10, HOX11, HOX11L2) were not detected in the TCRbeta-HOXA rearranged cases except for

deletion 9p21 and NOTCH1 activating mutations, which were present in 64 and 67%, respectively. In conclusion, this study defines TCRbeta-HOXA rearranged T-ALLs as a distinct cytogenetic subgroup by clinical, immunophenotypical and molecular genetic characteristics. *Leukemia* (2007) 21, 121-128. doi:10.1038/sj.leu.2404410; published online 12 October 2006.

FROHLING S., SCHOLL C., LEVINE R.L., LORIAUX M., BOGGON T.J., BERNARD O.A., BERGER R., DOHNER H., DOHNER K., EBERT B.L., TECKIE S., GOLUB T.R., JIANG J., SCHITTENHELM M.M., LEE B.H., GRIFFIN J.D., STONE R.M., HEINRICH M.C., DEININGER M.W., DRUKER B.J., GILLILAND D.G.

Identification of driver and passenger mutations of FLT3 by high-throughput DNA sequence analysis and functional assessment of candidate alleles.

Cancer Cell, 12 (6), 501-513, 2007

(Services cités : [E 0210](#))

Mutations in the juxtamembrane and kinase domains of FLT3 are common in AML, but it is not known whether alterations outside these regions contribute to leukemogenesis. We used a high-throughput platform to interrogate the entire FLT3 coding sequence in AML patients without known FLT3 mutations and experimentally tested the consequences of each candidate leukemogenic allele. This approach identified gain-of-function mutations that activated downstream signaling and conferred sensitivity to FLT3 inhibition and alleles that were not associated with kinase activation, including mutations in the catalytic domain. These findings support the concept that acquired mutations in cancer may not contribute to malignant transformation and underscore the importance of functional studies to distinguish "driver" mutations underlying tumorigenesis from biologically neutral "passenger" alterations.

LESSARD M., HELIAS C., STRUSKI S., PERRUSSON N., UETTWILLER F., MOZZICONACCI M.J., LAFAGE-POCHITALOFF M., DASTUGUE N., TERRE C., BRIZARD F., CORNILLET-LEFEBVRE P., MUGNERET F., BARIN C., HERRY A., LUQUET I., DESANGLES F., MICHAUX L., VERELLEN-DUMOULIN C., PERROT C., VAN DEN AKKER J., LESPINASSE J., ECLACHE V., BERGER R.

Fluorescence in situ hybridization analysis of 110 hematopoietic disorders with chromosome 5 abnormalities: do de novo and therapy-related myelodysplastic syndrome-acute myeloid leukemia actually differ ?

Cancer Genet. Cytogenet., 176 (1), 1-21, 2007

(Services cités : [E 0210](#))

A retrospective cytogenetic study of acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) was conducted by the Groupe Francophone de Cytogenétique Hematologique (GFCH) to evaluate the structural abnormalities of chromosome 5 associated with other chromosomal abnormalities, in particular of chromosome 7, in these pathologies. In all, 110 cases of AML/MDS were recruited based on the presence of chromosome 5 abnormalities under conventional cytogenetics and supplemented by a systematic fluorescence in situ hybridization study of chromosomes 5 and 7. The abnormalities of the long arm of chromosome 5 (5q) were deletions of various sizes and sometimes cryptic. The 5q abnormalities were associated with translocations in 54% of cases and were simple deletions in 46%. In 68% of cases, 5q deletions were associated with chromosome 7 abnormalities, and 90% of these presented a complex

karyotype. Of the 110 patients, 28 had a hematopoietic disorder secondary to chemotherapy, radiotherapy, or both. Among 82 patients with de novo AML/MDS, 63 were older than 60 years. Chromosomal abnormalities often associated hypodiploidy and chromosome 5 and 7 abnormalities in complex karyotypes, features resembling those of secondary hemopathies. Systematic investigation of the exposure to mutagens and oncogenes is thus essential to specify the factors potentially involved in MDS/AML with 5q abnormalities.

MALINGE S., BEN-ABDELALI R., SETTEGRANA C., RADFORD-WEISS I., DEBRE M., BELDJORD K., MACINTYRE E.A., VILLEVAL J.L., VAINCHENKER W., BERGER R., BERNARD O.A., DELABESSE E., PENARD-LACRONIQUE V.

Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia.

Blood, 109 (5), 2202-2204, 2007

(Services cités : E 0210, Histo-Embryologie - Cytogénétique, Laboratoire d'Hématologie, U768)

Activation of tyrosine kinase genes is a frequent event in human hematologic malignancies. Because gene activation could be associated with gene dysregulation, we attempted to screen for activating gene mutation based on high-level gene expression. We focused our study on the Janus kinase 2 (JAK2) gene in 90 cases of acute leukemia. This strategy led to the identification of a novel JAK2-acquired mutation in a patient with Down syndrome (DS) with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). This mutation involves a 5-amino acid deletion within the JH2 pseudokinase domain (JAK2DeltaREED). Expression of JAK2DeltaREED in Ba/F3 cells induced constitutive activation of the JAK-STAT pathway and growth factor-independent cell proliferation. These results highlight the JAK2 pseudokinase domain as an oncogenic hot spot and indicate that activation of the JAK-STAT pathway may contribute to lymphoid malignancies and hematologic disorders observed in children with DS.

PECQUET C., NYGA R., PENARD-LACRONIQUE V., SMITHGALL T.E., MURAKAMI H., REGNIER A., LASSOUED K., GOUILLEUX F.

The Src tyrosine kinase Hck is required for Tel-Abl- but not for Tel-Jak2-induced cell transformation.

Oncogene, 26 (11), 1577-1585, 2007

(Services cités : E 0210)

Tel-Abl and Tel-Jak2 are fusion proteins associated with human haematologic neoplasms. They possess constitutive tyrosine kinase activity and activate common downstream signalling pathways like Stat-5, PI3-K/Akt, Ras/MapK and NF-kappaB. In this study, we showed the specific requirement of Src family members for the Tel-Abl-mediated cell growth, activation of Stat5, PI3-K/Akt and Ras/MapK while dispensable for Tel-Jak2. Hck was found strongly phosphorylated in Tel-Abl-expressing Ba/F3 cells and sensitive to imatinib mesylate treatment, providing evidence that Hck is a target of Tel-Abl tyrosine kinase activity. Overexpression of a kinase dead form of Hck inhibits the proliferation of Ba/F3 cells expressing Tel-Abl as the phosphorylation of Akt and Erk1/2. These results argue for an important role of Hck in Tel-Abl oncogenic signalling.

RAFFEL G.D., MERCHER T., SHIGEMATSU H., WILLIAMS I.R., CULLEN D.E.,

AKASHI K., BERNARD O.A., GILLILAND D.G.

Ott1(Rbm15) has pleiotropic roles in hematopoietic development.

Proc. Nat. Acad. Sci. USA, 104 (14), 6001-6006, 2007

(Services cités : E 0210)

OTT1(RBM15) was originally described as a 5' translocation partner of the MAL(MKL1) gene in t(1,22)(p13;q13) infant acute megakaryocytic leukemia. OTT1 has no established physiological function, but it shares homology with the spen/Mint/SHARP family of proteins defined by three amino-terminal RNA recognition motifs and a carboxyl-terminal SPOC (Spen paralog and ortholog carboxyl-terminal) domain believed to act as a transcriptional repressor. To define the role of OTT1 in hematopoiesis and help elucidate the mechanism of t(1,22) acute megakaryocytic leukemia pathogenesis, a conditional allele of Ott1 was generated in mice. Deletion of Ott1 in adult mice caused a loss of peripheral B cells due to a block in pro/pre-B differentiation. There is myeloid and megakaryocytic expansion in spleen and bone marrow, an increase in the Lin(-)Sca-1(+)-Kit(+) compartment that includes hematopoietic stem cells, and a shift in progenitor fate toward granulocyte differentiation. These data show a requirement for Ott1 in B lymphopoiesis, and inhibitory roles in the myeloid, megakaryocytic, and progenitor compartments. The ability of Ott1 to affect hematopoietic cell fate and expansion in multiple lineages is a novel attribute for a spen family member and delineates Ott1 from other known effectors of hematopoietic development. It is plausible that dysregulation of Ott1-dependent hematopoietic developmental pathways, in particular those affecting the megakaryocyte lineage, may contribute to OTT1-MAL-mediated leukemogenesis.

**SIX E.M., BONHOMME D., MONTEIRO M., BELDJORD K., JURKOWSKA M.,
CORDIER-GARCIA C., GARRIGUE A., DAL-CORTIVO L., ROCHA B., FISCHER A.,
CAVAZZANA-CALVO M., ANDRE-SCHMUTZ I.**

A human postnatal lymphoid progenitor capable of circulating and seeding the thymus.

J. Exp. Med., 204 (13), 3085-3093, 2007

(Services cités : Biothérapie, E 0210, Immuno-Hématologie-Rhumatologie Pédiatriques, IRNEM, Laboratoire d'Hématologie, U591, U768)

Identification of a thymus-seeding progenitor originating from human bone marrow (BM) constitutes a key milestone in understanding the mechanisms of T cell development and provides new potential for correcting T cell deficiencies. We report the characterization of a novel lymphoid-restricted subset, which is part of the lineage-negative CD34(+)CD10(+) progenitor population and which is distinct from B cell-committed precursors (in view of the absence of CD24 expression). We demonstrate that these Lin(-)CD34(+)CD10(+)CD24(-) progenitors have a very low myeloid potential but can generate B, T, and natural killer lymphocytes and coexpress recombination activating gene 1, terminal deoxynucleotidyl transferase, PAX5, interleukin 7 receptor alpha, and CD3epsilon. These progenitors are present in the cord blood and in the BM but can also be found in the blood throughout life. Moreover, they belong to the most immature thymocyte population. Collectively, these findings unravel the existence of a postnatal lymphoid-polarized population that is capable of migrating from the BM to the thymus.

2006

ASNAFI V., RUBIO M.T., DELABESSE E., VILLAR E., DAVI F., DAMAJ G., HIRSCH I., DHEDIN N., VERNANT J.P., VARET B., BUZYN A., MACINTYRE E.

Prediction of relapse by day 100 BCR-ABL quantification after allogeneic stem cell transplantation for chronic myeloid leukemia.

Leukemia, 20 (5), 793-799, 2006

(Services cités : Hématologie Adulte, Laboratoire d'Hématologie, E 0210)

Chronic myeloid leukemia (CML) relapse after allogeneic stem cell transplantation (SCT) is a relatively frequent situation, which is correlated to disease status, time from diagnosis to transplant and T-cell depletion. We evaluated the potential for early minimal residual disease (MRD) BCR-ABL quantification to predict relapse of CML patients receiving allogeneic SCT. Minimal residual disease was analyzed by real-time quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) at day 100 (d100) in 38 patients with >1 year follow-up after conventional non-T-cell-depleted SCT. Normal ABL control values from 1724 follow-up blood samples were used to define an RQ-PCR amplifiability index and the limits of reliable use of BCR-ABL ratios. We then compared the 14 patients with a high-level d100 BCR-ABL/ABL ratio ($\geq 10^{-4}$) to that of the 24 patients with a negative/low-level ratio ($< 10^{-4}$). Despite being comparable for all classical parameters, the incidence of relapse was significantly higher in the high MRD group (11/14 (79%)) compared to that of the low/negative MRD group (7/24 (29%)) ($P=0.009$), with d100 MRD values representing an independent risk factor of relapse and disease-free survival, but not of overall survival, in multivariate analysis. These data should facilitate risk-adapted post-transplant immunosuppression and/or tyrosine kinase inhibitor therapy based on an early evaluation of MRD. *Leukemia* (2006) 20, 793-799. doi:10.1038/sj.leu.2404170; published online 16 March 2006.

BARDET V., COSTA L.D., ELIE C., MALINGE S., DEMUR C., TAMBURINI J., LEFEBVRE P.C., WITZ F., LIOURE B., JOURDAN E., PIGNEUX A., IFRAH N., ATTAL M., DREYFUS F., MAYEUX P., LACOMBE C., BENNACEUR-GRISCELLI A., BERNARD O.A., BOUSCARY D., RECHER C.

Nucleophosmin status may influence the therapeutic decision in de novo acute myeloid leukemia with normal karyotype.

Leukemia, 20 (9), 1644-1646, 2006

(Services cités : E 0210)

BERGER R., BUSSON M., DASTUGUE N., RADFORD-WEISS I., MICHAUX L., HAGEMEIJER A., QUILICHINI B., BENATTAR L., BERNARD O., ROMANA S.P.

Acute megakaryoblastic leukemia and loss of the RUNX1 gene.

Cancer Genet. Cytogenet., 164 (1), 71-73, 2006

(Services cités : E 0210, Histo-Embryologie & Cytogénétique)

Since the RUNX1 gene contributes to megakaryopoiesis and acquired trisomy 21 is the most frequent numerical chromosome anomaly in acute megakaryoblastic leukemia (AMLK), a systematic study of RUNX1 abnormalities was performed by fluorescence in situ hybridization in

AMLK patients. Four abnormalities were detected among 15 patients. One copy of RUNX1 was completely or partially lost in three patients and translocated onto Xq24 in the fourth. The possible consequences of RUNX1 haploinsufficiency are discussed.

BERGER R., BUSSON M., ROMANA S.P.

A cryptic chromosome 19 abnormality in a patient with Ph-positive acute lymphoblastic leukemia.

Cancer Genet. Cytogenet., 165 (1), 79-80, 2006

(Services cités : E 0210, Histo-Embryologie & Cytogénétique)

BERGER R., BUSSON M., BARANGER L., HELIAS C., LESSARD M., DASTUGUE N., SPELEMAN F.

Loss of the NPM1 gene in myeloid disorders with chromosome 5 rearrangements.

Leukemia, 20 (2), 319-321, 2006

(Services cités : E 0210)

The assignment with chromosome banding techniques of the breakpoints of the recurrent translocation t(3;5) which leads to NPM1/MLF1 gene fusion in myeloid malignancies has not been unequivocal. In order to assess whether this is due to uncertainty in interpretation of the observed banding pattern or whether it reflects true genomic heterogeneity, we decided to analyze the breakpoint positions using fluorescence in situ (FISH) techniques in eight patients with myeloid malignancies and rearrangements of chromosomes 3 and 5. In three patients, colocalization of the NPM1 and MLF1 spanning BACs was demonstrated and NPM1/MLF1 fusion shown by PCR in one while in the remaining cases breakpoints were located outside the NPM1 and MLF1 loci. Interestingly, loss of a copy of the NPM1 gene was found in three of these latter patients. This findings suggest that haploinsufficiency of NPM1 may play a role in subtypes of myelodysplasias and leukemias. *Leukemia* (2006) 20, 319-321. doi:10.1038/sj.leu.2404063; published online 8 December 2005.

BERGER R.

A recurrent mutation of the JAK2 gene in chronic myeloproliferative disorders.

Pathol. Biol., 54 (4), 182-184, 2006

(Services cités : E 0210)

BERGERON J., CLAPPIER E., CAUWELIER B., DASTUGUE N., MILLIEN C., DELABESSE E., BELDJORD K., SPELEMAN F., SOULIER J., MACINTYRE E., ASNAFI V.

HOXA cluster deregulation in T-ALL associated with both a TCRD-HOXA and a CALM-AF10 chromosomal translocation.

Leukemia, 20 (6), 1184-1187, 2006

(Services cités : E 0210, Laboratoire d'Hématologie)

BOURQUIN J.P., SUBRAMANIAN A., LANGEBRAKE C., REINHARDT D., BERNARD O., BALLERINI P., BARUCHEL A., CAVE H., DASTUGUE N., HASLE H., KASPERS G.L., LESSARD M., MICHAUX L., VYAS P., VAN WERING E., ZWAAN C.M., GOLUB T.R., ORKIN S.H.

Identification of distinct molecular phenotypes in acute megakaryoblastic leukemia by gene expression profiling.

Proc. Nat. Acad. Sci. USA, 103 (9), 3339-3344, 2006

(Services cités : E 0210)

Individuals with Down syndrome (DS) are predisposed to develop acute megakaryoblastic leukemia (AMKL), characterized by expression of truncated GATA1 transcription factor protein (GATA1s) due to somatic mutation. The treatment outcome for DS-AMKL is more favorable than for AMKL in non-DS patients. To gain insight into gene expression differences in AMKL, we compared 24 DS and 39 non-DS AMKL samples. We found that non-DS-AMKL samples cluster in two groups, characterized by differences in expression of HOX/TALE family members. Both of these groups are distinct from DS-AMKL, independent of chromosome 21 gene expression. To explore alterations of the GATA1 transcriptome, we used cross-species comparison with genes regulated by GATA1 expression in murine erythroid precursors. Genes repressed after GATA1 induction in the murine system, most notably GATA-2, MYC, and KIT, show increased expression in DS-AMKL, suggesting that GATA1s fail to repress this class of genes. Only a subset of genes that are up-regulated upon GATA1 induction in the murine system show increased expression in DS-AMKL, including GATA1 and BACH1, a probable negative regulator of megakaryocytic differentiation located on chromosome 21. Surprisingly, expression of the chromosome 21 gene RUNX1, a known regulator of megakaryopoiesis, was not elevated in DS-AMKL. Our results identify relevant signatures for distinct AMKL entities and provide insight into gene expression changes associated with these related leukemias.

CHAPIRO E., DELABESSE E., ASNAFI V., MILLIEN C., DAVI F., NUGENT E., BELDJORD K., HAFERLACH T., GRIMWADE D., MACINTYRE E.A.

Expression of T-lineage-affiliated transcripts and TCR rearrangements in acute promyelocytic leukemia: implications for the cellular target of t(15;17).

Blood, 108 (10), 3484-3493, 2006

(Services cités : E 0210, Laboratoire d'Hématologie)

Acute promyelocytic leukemia (APL) is the most differentiated form of acute myeloid leukemia (AML) and has generally been considered to result from transformation of a committed myeloid progenitor. Paradoxically, APL has long been known to express the T-cell lymphoid marker, CD2. We searched for other parameters indicative of T-cell lymphoid specification in a cohort of 36 APL cases, revealing a frequent but asynchronous T-cell lymphoid program most marked in the hypogranular variant (M3v) subtype, with expression of PTCRA, sterile TCRA, and TCRG transcripts and TCRG rearrangement in association with sporadic cytoplasmic expression of CD3 or TdT proteins. Gene-expression profiling identified differentially expressed transcription factors that have been implicated in lymphopoiesis. These data carry implications for the hematopoietic progenitor targeted by the PML-RARA oncoprotein in APL and are suggestive of a different cellular origin for classic hypergranular (M3) and variant forms of the disease. They are also consistent with the existence and subsequent transformation of progenitor populations with lymphoid/myeloid potential.

CHAPIRO E., RUSSELL L., RADFORD-WEISS I., BASTARD C., LESSARD M., STRUSKI S., CAVE H., FERT-FERRER S., BARIN C., MAAREK O., DELLA-VALLE V., STREFFORD J.C., BERGER R., HARRISON C.J., BERNARD O.A., NGUYEN-KHAC F.

Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia.

Blood, 108 (10), 3560-3563, 2006

(Services cités : E 0210, Histo-Embryologie & Cytogénétique)

Subtle variation in the expression or function of a small group of transcription factors can drive leukemogenesis. The CEBPA protein is known to regulate the balance between cell proliferation and differentiation during early hematopoietic development and myeloid differentiation. In human myeloid leukemia, CEBPA is frequently inactivated by mutation and indirect and posttranslational mechanisms, in keeping with tumor suppressor properties. We report that CEBPA is activated by juxtaposition to the immunoglobulin gene enhancer upon its rearrangement with the immunoglobulin heavy-chain locus in precursor B-cell acute lymphoblastic leukemia harboring t(14;19)(q32;q13). Overexpression of apparently normal CEBPA RNA or protein was observed in 6 patients. These data indicate that CEBPA may exhibit oncogenic as well as tumor suppressor properties in human leukemogenesis.

DELNATTE C., SANLAVILLE D., MOUGENOT J.F., VERMEESCH J.R., HOUDAYER C., BLOIS M.C., GENEVIEVE D., GOULET O., FRYNS J.P., JAUBERT F., VEKEMANS M., LYONNET S., ROMANA S., ENG C., STOPPA-LYONNET D.

Contiguous Gene Deletion within Chromosome Arm 10q Is Associated with Juvenile Polyposis of Infancy, Reflecting Cooperation between the BMPR1A and PTEN Tumor-Suppressor Genes. *Amer. J. Hum. Genet.*, 78 (6), 1066-1074, 2006

(Services cités : Anatomo-Pathologie, Département de Pédiatrie, E 0210, Génétique Médicale Pédiatrique)

We describe four unrelated children who were referred to two tertiary referral medical genetics units between 1991 and 2005 and who are affected with juvenile polyposis of infancy. We show that these children are heterozygous for a germline deletion encompassing two contiguous genes, PTEN and BMPR1A. We hypothesize that juvenile polyposis of infancy is caused by the deletion of these two genes and that the severity of the disease reflects cooperation between these two tumor-suppressor genes.

JEANDIDIER E., DASTUGUE N., MUGNERET F., LAFAGE-POCHITALOFF M., MOZZICONACCI M.J., HERENS C., MICHAUX L., VERELLEN-DUMOULIN C., TALMANT P., CORNILLET-LEFEBVRE P., LUQUET I., CHARRIN C., BARIN C., COLLONGE-RAME M.A., PEROT C., VAN DEN AKKER J., GREGOIRE M.J., JONVEAUX P., BARANGER L., ECLACHE-SAUDREAU V., PAGES M.P., CABROL C., TERRE C., BERGER R.

Abnormalities of the long arm of chromosome 21 in 107 patients with hematopoietic disorders: a collaborative retrospective study of the Groupe Francais de Cytogenetique Hematologique.

Cancer Genet. Cytogenet., 166 (1), 1-11, 2006

(Services cités : E 0210)

Chromosome 21 is frequently rearranged in hematopoietic malignancies. In order to detect new chromosomal aberrations, the Groupe Francais de Cytogenetique Hematologique collected a series of 107 patients with various hematologic disorders and acquired structural abnormalities of the long arm of chromosome 21. The abnormalities were subclassified into 10 groups, according to the location of the 21q breakpoint and the type of abnormality. Band 21q22 was implicated in 72 patients (excluding duplications, triplications, and amplifications). The involvement of the RUNX1 gene was confirmed in 10 novel translocations, but the gene partners were not identified. Eleven novel translocations rearranging band 21q22 with bands 1q25, 2p21, 2q37, 3p21, 3p23, 4q31, 6p24 approximately p25, 6p12, 7p15, 16p11, and 18q21 were detected. Rearrangements of band 21q11 and 21q21 were detected in six novel translocations with 5p15, 6p21, 15q21, 16p13,

and 20q11 and with 1p33, 3q27, 5p14, 11q11, and 14q11, respectively. Duplications, triplications, amplifications, and isodicentric chromosomes were detected in eight, three, eight, and three patients, respectively. The present study shows both the wide distribution of the breakpoints on the long arm of chromosome 21 in hematopoietic malignancy and the diversity of the chromosomal rearrangements and the hematologic disorders involved. The findings invite further investigation of the 21q abnormalities to detect their associated molecular rearrangements.

KARRMAN K., ANDERSSON A., BJORGVINSDOTTIR H., STROMBECK B., LASSEN C., OLOFSSON T., NGUYEN-KHAC F., BERGER R., BERNARD O., FIORETOS T., JOHANSSON B.

Deregulation of cyclin D2 by juxtaposition with T-cell receptor alpha/delta locus in t(12;14)(p13;q11)-positive childhood T-cell acute lymphoblastic leukemia.

Eur. J. Haematol., 77 (1), 27-34, 2006

(Services cités : E 0210)

Objectives: The t(12;14)(p13;q11) - a recurrent translocation in childhood T-cell acute lymphoblastic leukemia (T-ALL) - has very recently been molecularly characterized in one case, which displayed overexpression of the cyclin D2 gene (CCND2). Patients and methods: We have characterized two pediatric t(12;14)-positive T-ALLs using fluorescence in situ hybridization (FISH), cDNA microarray, and real-time polymerase chain reaction (PCR). Results: FISH revealed breakpoints (BPs) in the T-cell receptor alpha/delta locus (14q11) and in the vicinity of the CCND2 gene at 12p13. To investigate the expression of genes in 12p13, cDNA microarray analysis was performed. Expression data for eight genes, including CCND2, surrounding the 12p BP were compared with those in other T-ALLs. The t(12;14)-positive T-ALL displayed an increased expression of CCND2 compared to the controls, whereas the expression of the other genes was similar in all T-ALLs. Expression of CCND2 and two additional genes (PARP11 and FGF23), close to the 12p BP, was investigated with real-time PCR of the two t(12;14)-positive cases and four controls. Neither PARP11 nor FGF23 displayed expression differences among the T-ALLs, whereas CCND2 was clearly overexpressed in both t(12;14)-positive cases as compared to the mean expression level in the controls. Conclusion: We have confirmed, in two additional cases, that the recurrent T-ALL-associated t(12;14) results in overexpression of cyclin D2. The t(12;14) is the first neoplasia-associated translocation shown to result in overexpression of cyclin D2. Furthermore, it is the first example of a T-cell neoplasm with a targeted deregulation of a member of a cyclin-encoding gene family.

LHERMITTE L., de LABARTHE A., DUPRET C., LAPILLONNE H., MILLIEN C., LANDMAN-PARKER J., HERMINE O., BARUCHEL A., SIGAUX F., MACINTYRE E., ASNAFI V.

Most immature T-ALLs express Ra-IL3 (CD123): possible target for DT-IL3 therapy.

Leukemia, 20 (10), 1908-1910, 2006

(Services cités : Hématologie Adulte, Laboratoire d'Hématologie, E 0210)

MALINGE S., MONNI R., BERNARD O., PENARD-LACRONIQUE V.

Activation of the NF-kappaB pathway by the leukemogenic TEL-Jak2 and TEL-Abl fusion proteins leads to the accumulation of antiapoptotic IAP proteins and involves IKKalpha.

Oncogene, 25 (25), 3589-3597, 2006

(Services cités : E 0210)

Abnormal activation of tyrosine kinases and of signaling pathways they control plays a critical

role in the neoplastic process of human hematopoietic malignancy. The nuclear factor-kappaB (NF-kappaB) pathway is one of the signalings activated by the TEL-Jak2 and TEL-Abl oncoproteins and required for their antiapoptotic activity. To define the signal relay responsible for this activation, we used mouse embryonic fibroblast (MEF) cells and observed that TEL-Jak2- and TEL-Abl-mediated NF-kappaB induction was abolished in cells lacking the IKKalpha kinase (IKKalpha) but not in IKKbeta(-/-) cells. Similar observations were performed with oncogenic forms of the FMS-like tyrosine kinase 3 (Flt-3) involved in the pathogenesis of one-third of acute myeloid leukemias. Rescue of TEL-Jak2-mediated NF-kappaB activation was obtained with a kinase-proficient form of IKKalpha in IKKalpha(-/-) MEF. Hematopoietic cells transformed by TEL-Jak2 and TEL-Abl showed sustained IKKalpha activity without promotion of NF-kappaB2/p100 processing, generally associated to IKKalpha functions. Furthermore, IAP1, IAP2 and XIAP, which are central regulators of the NF-kappaB-mediated survival pathway, were highly expressed in cells transformed by these oncoproteins. Our results indicate that these oncogenic tyrosine kinases preferentially use an IKKalpha-dependent mechanism to induce a persistent NF-kappaB activity and allow the production of antiapoptotic effectors that participate to their leukemogenic properties. *Oncogene* (2006) 25, 3589-3597. doi:10.1038/sj.onc.1209390; published online 23 January 2006.

MERCHER T., WERNIG G., MOORE S.A., LEVINE R.L., GU T.L., FROHLING S., CULLEN D., POLAKIEWICZ R.D., BERNARD O.A., BOGGON T.J., LEE B.H., GILLILAND D.G.

JAK2T875N is a novel activating mutation that results in myeloproliferative disease with features of megakaryoblastic leukemia in a murine bone marrow transplantation model.

Blood, 108 (8), 2770-2779, 2006

(Services cités : [E 0210](#))

Acute megakaryoblastic leukemia (AMKL) is a subtype of acute myeloid leukemia associated with a poor prognosis. However, there are relatively few insights into the genetic etiology of AMKL. We developed a screening assay for mutations that cause AMKL, based on the hypothesis that constitutive activation of STAT5 would be a biochemical indicator of mutation in an upstream effector tyrosine kinase. We screened human AMKL cell lines for constitutive STAT5 activation, and then used an approach combining mass spectrometry identification of tyrosine phosphorylated proteins and growth inhibition in the presence of selective small molecule tyrosine kinase inhibitors that would inform DNA sequence analysis of candidate tyrosine kinases. Using this strategy, we identified a new JAK2T875N mutation in the AMKL cell line CHRF-288-11. JAK2T875N is a constitutively activated tyrosine kinase that activates downstream effectors including STAT5 in hematopoietic cells in vitro. In a murine transplant model, JAK2T875N induced a myeloproliferative disease characterized by features of AMKL, including megakaryocytic hyperplasia in the spleen; impaired megakaryocyte polyploidization; and increased reticulin fibrosis of the bone marrow and spleen. These findings provide new insights into pathways and therapeutic targets that contribute to the pathogenesis of AMKL.

NGUYEN-KHAC F., DELLA VALLE V., LOPEZ R.G., RAVET E., MAUCHAUFFE M., FRIEDMAN A.D., HUANG L.E., FICHELSON S., GHYSDAEL J., BERNARD O.A.

Functional analyses of the TEL-ARNT fusion protein underscores a role for oxygen tension in hematopoietic cellular differentiation.

Oncogene, 25 (35), 4840-4847, 2006

(Services cités : [E 0210](#))

The transcription factor hypoxia inducible factor 1 (HIF1), an HIF1alpha-aryl hydrocarbon receptor nuclear translocator (ARNT) dimeric factor, is essential to the cellular response to hypoxia. We described a t(1;12)(q21;p13) chromosomal translocation in human acute myeloblastic leukemia that involves the translocated Ets leukemia (TEL/ETV6) and the ARNT genes and results in the expression of a TEL-ARNT fusion protein. Functional studies show that TEL-ARNT interacts with HIF1alpha and the complex binds to consensus hypoxia response element. In low oxygen tension conditions, the HIF1alpha/TEL-ARNT complex does not activate transcription but exerts a dominant-negative effect on normal HIF1 activity. Differentiation of normal human CD34+ progenitors cells along all the erythrocytic, megakaryocytic and granulocytic pathways was accelerated in low versus high oxygen tension conditions. Murine 32Dcl3 myeloid cells also show accelerated granulocytic differentiation in low oxygen tension in response to granulocyte colony-stimulating factor. Interestingly, stable expression of the TEL-ARNT in 32Dcl3 subclones resulted in impaired HIF1-mediated transcriptional response and inhibition of differentiation enhancement in hypoxic conditions. Taken together, our results underscore the role of oxygen tension in the modulation of normal hematopoietic differentiation, whose targeting can participate in human malignancies. *Oncogene* (2006) 25, 4840-4847. doi:10.1038/sj.onc.1209503; published online 20 March 2006.

ROMANA S.P., RADFORD-WEISS I., BEN ABDELALI R., SCHLUTH C., PETIT A., DASTUGUE N., TALMANT P., BILHOU-NABERA C., MUGNERET F., LAFAGE-POCHITALOFF M., MOZZICONACCI M.J., ANDRIEU J., LAI J.L., TERRE C., RACK K., CORNILLET-LEFEBVRE P., LUQUET I., NADAL N., NGUYEN-KHAC F., PEROT C., VAN DEN AKKER J., FERT-FERRER S., CABROL C., CHARRIN C., TIGAUD I., POIREL H., VEKEMANS M., BERNARD O.A., BERGER R.

NUP98 rearrangements in hematopoietic malignancies: a study of the Groupe Francophone de Cytogenetique Hematologique.

Leukemia, 20 (4), 696-706, 2006

(Services cités : E 0210, Histo-Embryologie & Cytogénétique)

The NUP98 gene is fused with 19 different partner genes in various human hematopoietic malignancies. In order to gain additional clinico-hematological data and to identify new partners of NUP98, the Groupe Francophone de Cytogenetique Hematologique (GFCH) collected cases of hematological malignancies where a 11p15 rearrangement was detected. Fluorescence in situ hybridization (FISH) analysis showed that 35% of these patients (23/66) carried a rearrangement of the NUP98 locus. Genes of the HOXA cluster and the nuclear-receptor set domain (NSD) genes were frequently fused to NUP98, mainly in de novo myeloid malignancies whereas the DDX10 and TOP1 genes were equally rearranged in de novo and in therapy-related myeloid proliferations. Involvement of ADD3 and C6ORF80 genes were detected, respectively, in myeloid disorders and in T-cell acute lymphoblastic leukemia (T-ALL), whereas the RAP1GDS1 gene was fused to NUP98 in T-ALL. Three new chromosomal breakpoints: 3q22.1, 7p15 (in a localization distinct from the HOXA locus) and Xq28 were detected in rearrangements with the NUP98 gene locus. The present study as well as a review of the 73 cases previously reported in the literature allowed us to delineate some chromosomal, clinical and molecular features of patients carrying a NUP98 gene rearrangements. *Leukemia* (2006) 20, 696-706. doi:10.1038/sj.leu.2404130; published online 9 February 2006.

SANLAVILLE D., DELNATTE C., MOUGENOT J.F., VERMEESCH J.R., HOUDAYER C., de BLOIS M.C., GENEVIEVE D., GOULET O., FRYNS J.P., JAUBERT F.,

VEKEMANS M., LYONNET S., ROMANA S., ENG C., STOPPA-LYONNET D.

Reply to salviati et al.

Amer. J. Hum. Genet., 79 (3), 596-597, 2006

(Services cités : Anatomo-Pathologie, E 0210, Génétique Médicale Pédiatrique)

SINDT A., DEAU B., BRAHIM W., STAAL A., VISANICA S., VILLARESE P., RAULT J.P., MACINTYRE E., DELABESSE E.

Acute monocytic leukemia with coexpression of minor BCR-ABL1 and PICALM-MLLT10 fusion genes along with overexpression of HOXA9.

Genes Chromosome Cancer, 45 (6), 575-582, 2006

(Services cités : E 0210, Hématologie Adulte, Laboratoire d'Hématologie)

The t(9;22)(q34;q11) translocation occurs in chronic myeloid leukemia (CML) and adult B-cell acute lymphoblastic leukemia (ALL), leading to fusion of BCR to ABL1 and constitutive activation of ABL1 tyrosine kinase activity. The main BCR-ABL1 breakpoints result in P190 BCR-ABL1 or P210 BCR-ABL1 fusion proteins. The latter is found in almost all cases of CML and in one third of the cases of t(9;22)-positive adult B-ALL. P190 BCR-ABL1 is found in the remaining two thirds of t(9;22)-positive adult B-ALL cases but only exceptionally in CML. We describe here the first case of t(9;22)(q34;q11) associated with t(10;11)(p13;q14) in acute monocytic leukemia. The recurrent t(10;11)(p13;q14) translocation, usually found in acute myeloid leukemia (AML) and T-ALL, merges PICALM to MLLT10. RT-PCR enabled identification of PICALM-MLLT10 and BCR-ABL1 e1-a2 fusion transcripts; in the context of chronic and acute myeloid leukemia, the latter usually has a monocytic presentation. We also identified overexpression of HOXA9, a gene essential to myeloid differentiation that is expressed in PICALM-MLLT10 and MLL-rearranged acute leukemias. This case fits with and extends a recently proposed multistage AML model in which constitutive activation of tyrosine kinases by mutations (BCR-ABL1) are associated with deregulation of transcription factors central to myeloid differentiation (HOXA9 secondary to PICALM-MLLT10).

SU X., DRABKIN H., CLAPPIER E., MORGADO E., BUSSON M., ROMANA S., SOULIER J., BERGER R., BERNARD O.A., LAVAU C.

Transforming potential of the T-cell acute lymphoblastic leukemia-associated homeobox genes HOXA13, TLX1, and TLX3.

Genes Chromosome Cancer, 45 (9), 846-855, 2006

(Services cités : E 0210)

The importance of HOXA genes in T-cell acute lymphoblastic leukemia (T-ALL) has recently been recognized. We report a novel chromosomal translocation in a T-ALL patient that maps upstream of the HOXA13 gene and downstream of the BCL11B/CTIP2 locus. Analysis of HOXA gene transcription demonstrated massive expression of HOXA13, whereas the other HOXA genes were unaffected. A genomic rearrangement of the HOXA locus associated with exclusive expression of HOXA13 was observed in a second patient. This situation resembles chromosomal translocations activating genes of the TLX/HOX11 family in T-ALLs. To compare the leukemogenic properties of HOXA13 to that of TLX proteins, cohorts of lethally irradiated mice were transplanted with bone marrow transduced with a retroviral vector expressing TLX3 or HOXA13. Cells transduced with TLX3 or HOXA13 could not be detected in the peripheral blood of mice post-transplantation and none of the mice developed malignancies. Cotransduction of the HOX cofactor MEIS1 with TLX3 or HOXA13 did not alter this outcome. However, in a myeloid clonogenic assay HOXA13 and TLX3 extended the proliferation of progenitors similarly to what

was observed for TLX1. Altogether, our results strongly suggest the absolute requirement for cooperative events in association with homeobox gene up-regulation to induce T-cell leukemogenesis. (c) 2006 Wiley-Liss, Inc.

SU X.Y., DELLA-VALLE V., ANDRE-SCHMUTZ I., LEMERCIER C., RADFORD-WEISS I., BALLERINI P., LESSARD M., LAFAGE-POCHITALOFF M., MUGNERET F., BERGER R., ROMANA S.P., BERNARD O.A., PENARD-LACRONIQUE V.

HOX11L2/TLX3 is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32).

Blood, 108 (13), 4198-4201, 2006

(Services cités : E 0210, Histo-Embryologie & Cytogénétique)

The t(5;14)(q35;q32) chromosomal translocation is specifically observed in up to 20% of childhood T-cell acute lymphoblastic leukemia (T-ALL). It affects the BCL11B/CTIP2 locus on chromosome 14 and the RANBP17-TLX3/HOX11L2 region on chromosome 5. It leads to ectopic activation of TLX3/HOX11L2. To investigate the reasons of the association between t(5;14) and T-ALL, we isolated the translocation breakpoints in 8 t(5;14) patients. Sequence analyses did not involve recombinase activity in the genesis of the translocation. We used DNase1 hypersensitive experiments to locate transcriptional regulatory elements downstream of BCL11B. By transient transfection experiments, 2 of the 6 regions demonstrated cis-activation properties in T cells and were also effective on the TLX3 promoter. Our data indicate that the basis of the specific association between t(5;14) and T-ALL lies on the juxtaposition of TLX3 to long-range cis-activating regions active during T-cell differentiation.

2005

BALLERINI P., BUSSON M., FASOLA S., VAN DEN AKKER J., LAPILLONNE H., ROMANA S.P., MARYNEN P., BERNARD O., LANDMAN-PARKER J., BERGER R.

NUP214-ABL1 amplification in t(5;14)/HOX11L2-positive ALL present with several forms and may have a prognostic significance.

Leukemia, 19 (3), 468-470, 2005

(Services cités : E 0210, Histo-Embryologie & Cytogénétique)

DIK W.A., BRAHIM W., BRAUN C., ASNAFI V., DASTUGUE N., BERNARD O.A., VAN DONGEN J.J., LANGERAK A.W., MACINTYRE E.A., DELABESSE E.

CALM-AF10+ T-ALL expression profiles are characterized by overexpression of HOXA and BMI1 oncogenes.

Leukemia, 19 (11), 1948-1957, 2005

(Services cités : E 0210, Hématologie Adulte)

The t(10;11)(p13;q14-21) is found in T-ALL and acute myeloid leukemia and fuses CALM (Clathrin-Assembly protein-like Lymphoid-Myeloid leukaemia gene) to AF10. In order to gain insight into the transcriptional consequences of this fusion, microarray-based comparison of CALM-AF10+ vs CALM-AF10- T-ALL was performed. This analysis showed upregulation of HOXA5, HOXA9, HOXA10 and BMI1 in the CALM-AF10+ cases. Microarray results were validated by quantitative RT-PCR on an independent group of T-ALL and compared to mixed lineage leukemia-translocated acute leukemias (MLL-t AL). The overexpression of HOXA genes was associated with overexpression of its cofactor MEIS1 in CALM-AF10+ T-ALL, reaching levels of expression similar to those observed in MLL-t AL. Consequently, CALM-AF10+ T-ALL and MLL-t AL share a specific HOXA overexpression, indicating they activate common

oncogenic pathways. In addition, BMI1, located close to AF10 breakpoint, was overexpressed only in CALM-AF10+ T-ALL and not in MLL-t AL. BMI1 controls cellular proliferation through suppression of the tumor suppressors encoded by the CDKN2A locus. This locus, often deleted in T-ALL, was conserved in CALM-AF10+ T-ALL. This suggests that decreased CDKN2A activity, as a result of BMI1 overexpression, contributes to leukemogenesis in CALM-AF10+ T-ALL. We propose to define a HOXA+ leukemia group composed of at least MLL-t, CALM-AF10 and HOXA-t AL, which may benefit from adapted management. *Leukemia* (2005) 19, 1948-1957. doi:10.1038/sj.leu.2403891; published online 18 August 2005.

DUPRET C., ASNAFI V., LEBOEUF D., MILLIEN C., BEN ABDELALI R., PREUDHOMME C., BELDJORD K., DELABESSE E., MACINTYRE E.

IgH/TCR rearrangements are common in MLL translocated adult AML and suggest an early T/myeloid or B/myeloid maturation arrest, which correlates with the MLL partner.

Leukemia, 19 (12), 2337-2338, 2005

(Services cités : E 0210, Laboratoire d'Hématologie)

EL-BCHIRI J., BUHARD O., PENARD-LACRONIQUE V., THOMAS G., HAMELIN R., DUVAL A.

Differential nonsense mediated decay of mutated mRNAs in mismatch repair deficient colorectal cancers.

Hum. Mol. Genet., 14 (16), 2435-2442, 2005

(Services cités : E 0210)

The nonsense-mediated decay (NMD) system normally targets mRNAs with premature termination codons (PTCs) for rapid degradation. We investigated for a putative role of NMD in cancers with microsatellite instability (MSI-H cancers), because numerous mutant mRNAs containing PTC are generated in these tumors as a consequence of their mismatch repair deficiency. Using a quantitative RT-PCR approach in a large series of colorectal cancer cell lines, we demonstrate a significantly increased rate of degradation of mutant mRNAs containing a PTC compared with wild-type. A specific siRNA strategy was used to inhibit RENT-1 and/or RENT-2 activity, two major genes in the NMD system. This allowed us to show that increased degradation of PTC-containing mRNAs in MSI-H tumors was partly dependent upon NMD activity. The efficiency of NMD for the degradation of mutant mRNAs from target genes was highly variable in these cancers. NMD degraded some of them (TGF β 2, MSH3, GRK4), although allowing the persistent expression of others (BAX, TCF-4). This is of particular interest within the context of a proposed conservation of biological activity for the corresponding mutated proteins. We thus propose that NMD might play an important role in the selection of target gene mutations with a functional role in MSI-H carcinogenesis.

GARCON L., LIBURA M., DELABESSE E., VALENSI F., ASNAFI V., BERGER C., SCHMITT C., LEBLANC T., BUZYN A., MACINTYRE E.

DEK-CAN molecular monitoring of myeloid malignancies could aid therapeutic stratification.

Leukemia, 19 (8), 1338-1344, 2005

(Services cités : E 0210, Hématologie Adulte, Laboratoire d'Hématologie)

The t(6;9)(p23;q34) is a recurrent chromosomal abnormality observed in 1% of acute myelogenous leukemia (AML), which generates a fusion transcript between DEK and CAN/NUP214 genes. We used a DEK-CAN real-time quantitative (RQ)-PCR strategy to analyze 79 retrospective and prospective samples from 12 patients. Five patients reached DEK-CAN

negativity (sensitivity 10(-5)); all underwent early allogeneic hematopoietic stem cell transplantation (median 5.5 months from diagnosis) with some demonstrating molecular positivity at the time of allograft. All four cases in CCR with adequate follow-up (median 18.5 months, range 13-95) demonstrate persistent molecular negativity, whereas all seven patients with persistent DEK-CAN positivity died at a median of 12 months from diagnosis (range 7-27). We conclude that DEK-CAN molecular monitoring by RQ-PCR in t(6;9) malignancies is a useful tool for individual patient management and that molecular negativity is indispensable for survival, but should not be a prerequisite for allografting in this rare, poor prognosis, subset of AML. *Leukemia* (2005) 19, 1338-1344. doi:10.1038/sj.leu.2403835; published online 23 June 2005.

HAYETTE S., CORNILLET-LEFEBVRE P., TIGAUD I., STRUSKI S., FORISSIER S., BERCHET A., DOLL D., GILLOT L., BRAHIM W., DELABESSE E., MAGAUD J.P., RIMOKH R.

AF4p12, a human homologue to the furry gene of *Drosophila*, as a novel MLL fusion partner. *Cancer Res.*, 65 (15), 6521-6525, 2005

(Services cités : [E 0210](#))

More than 35 different partner genes with the mixed lineage leukemia (MLL) gene have been cloned from leukemia cells with translocations involving chromosome 11 band q23. In this study, we report on a novel fusion partner of the MLL gene, AF4p12, which we have identified as the human homologue to the furry gene of *Drosophila*. AF4p12, highly conserved in evolution, encodes a large protein of 3,105 amino acids. The expression of AF4p12 has been preferentially detected in colon, placenta, and brain tissues and in tumor cells of lymphoid origin. We show that the t(4;11)(p12;q23) translocation results in the creation of a chimeric RNA encoding a putative fusion protein containing 1,362 amino acids from the NH₂-terminal part of MLL and 712 amino acids from the COOH-terminal part of AF4p12. FLT3 and HOXA9 genes are overexpressed in this leukemia. We found that the COOH-terminal part of AF4p12 fused to MLL contains a leucine zipper motif and exhibits transcriptional activation properties when fused to Gal4 DNA-binding domains in transient transfection assays. The AF4p12 fragment fused to MLL may contribute to the oncogenic activation of MLL, possibly through specific recruitment of the transcriptional machinery.

HIRIART E., GRUFFAT H., BUISSON M., MIKAEKIAN I., KEPPLER S., MERESSE P., MERCHER T., BERNARD O.A., SERGEANT A., MANET E.

Interaction of the Epstein-Barr virus mRNA export factor EB2 with human Spen proteins SHARP, OTT1, and a novel member of the family, OTT3, links Spen proteins with splicing regulation and mRNA export.

J. Biol. Chem., 280 (44), 36935-36945, 2005

(Services cités : [E 0210](#))

The Epstein-Barr virus early protein EB2 (also called BMLF1, Mta, or SM), a protein absolutely required for the production of infectious virions, shares properties with mRNA export factors. By using a yeast two-hybrid screen, we have identified the human protein OTT3 as an EB2-interacting factor. OTT3 is a new member of the Spen (split end) family of proteins (huSHARP, huOTT1, DmSpen, and muMINT), which are characterized by several N-terminal RNA recognition motifs and a highly conserved C-terminal SPOC (Spen Paralog and Ortholog C-terminal) domain that, in the case of SHARP, has been shown to interact with SMRT/NCOR corepressors. OTT3 is ubiquitously expressed as a 120-kDa protein. Transfected OTT3 is a

nonshuttling nuclear protein that co-localizes with co-transfected EB2. We also showed that EB2 interacts with the SPOC domains of both OTT1 and SHARP proteins. Although the OTT3 interaction domain maps within the 40 N-terminal amino acids of EB2, OTT1 and SHARP interact within the C-terminal half of the protein. Furthermore, we demonstrated that the capacity of the OTT3 and OTT1 SPOC domains to interact with SMRT and repress transcription is far weaker than that of SHARP. Thus there is no evidence for a role of OTT3 in transcriptional regulation. Most interestingly, however, we have found that OTT3 has a role in splicing regulation; OTT3 represses accumulation of the alternatively spliced beta-thalassemia mRNAs, but it has no effect on the beta-globin constitutively spliced mRNA. Thus our results suggested a new function for Spen proteins related to mRNA export and splicing.

JAMES C., UGO V., LE COUEDIC J.P., STAERK J., DELHOMMEAU F., LACOUT C., GARCON L., RASLOVA H., BERGER R., BENNACEUR-GRISCELLI A., VILLEVAL J.L., CONSTANTINESCU S.N., CASADEVALL N., VAINCHENKER W.

A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera.

Nature, 434 (7037), 1144-1148, 2005

(Services cités : [E 0210](#))

Myeloproliferative disorders are clonal haematopoietic stem cell malignancies characterized by independency or hypersensitivity of haematopoietic progenitors to numerous cytokines. The molecular basis of most myeloproliferative disorders is unknown. On the basis of the model of chronic myeloid leukaemia, it is expected that a constitutive tyrosine kinase activity could be at the origin of these diseases. Polycythaemia vera is an acquired myeloproliferative disorder, characterized by the presence of polycythaemia diversely associated with thrombocytosis, leukocytosis and splenomegaly. Polycythaemia vera progenitors are hypersensitive to erythropoietin and other cytokines. Here, we describe a clonal and recurrent mutation in the JH2 pseudo-kinase domain of the Janus kinase 2 (JAK2) gene in most (> 80%) polycythaemia vera patients. The mutation, a valine-to-phenylalanine substitution at amino acid position 617, leads to constitutive tyrosine phosphorylation activity that promotes cytokine hypersensitivity and induces erythrocytosis in a mouse model. As this mutation is also found in other myeloproliferative disorders, this unique mutation will permit a new molecular classification of these disorders and novel therapeutical approaches.

KOMURA E., TONETTI C., PENARD-LACRONIQUE V., CHAGRAOUI H., LACOUT C., LECOUEDIC J.P., RAMEAU P., DEBILI N., VAINCHENKER W., GIRAUDIER S.

Role for the nuclear factor kappaB pathway in transforming growth factor-beta1 production in idiopathic myelofibrosis: possible relationship with FK506 binding protein 51 overexpression.

Cancer Res., 65 (8), 3281-3289, 2005

(Services cités : [E 0210](#))

The release of transforming growth factor-beta1 (TGF-beta1) in the bone marrow microenvironment is one of the main mechanisms leading to myelofibrosis in murine models and probably in the human idiopathic myelofibrosis (IMF). The regulation of TGF-beta1 synthesis is poorly known but seems regulated by nuclear factor kappaB (NF-kappaB). We previously described the overexpression of an immunophilin, FK506 binding protein 51 (FKBP51), in IMF megakaryocytes. Gel shift and gene assays show that FKBP51's overexpression in a factor-dependent hematopoietic cell line, induces a sustained NF-kappaB activation after cytokine deprivation. This activation correlates with a low level of IkappaBalpha. A spontaneous activation of NF-kappaB was also detected in proliferating megakaryocytes and in circulating

CD34(+) patient cells. In normal cells, NF-kappaB activation was only detected after cytokine treatment. The expression of an NF-kappaB superrepressor in FKBP51 overexpressing cells and in derived megakaryocytes from CD34(+) of IMF patients revealed that NF-kappaB activation was not involved in the resistance to apoptosis after cytokine deprivation of these cells but in TGF-beta1 secretion. These results highlight the importance of NF-kappaB's activation in the fibrosis development of this disease. They also suggest that FKBP51's overexpression in IMF cells could play an important role in the pathogenesis of this myeloproliferative disorder.

LEVINE R.L., LORIAUX M., HUNTLY B.J., LOH M.L., BERAN M., STOFFREGEN E., BERGER R., CLARK J.J., WILLIS S.G., NGUYEN K.T., FLORES N.J., ESTEY E., GATTERMANN N., ARMSTRONG S., LOOK A.T., GRIFFIN J.D., BERNARD O.A., HEINRICH M.C., GILLILAND D.G., DRUKER B., DEININGER M.W.

The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia.

Blood, 106 (10), 3377-3379, 2005

(Services cités : [E 0210](#))

Activating mutations in tyrosine kinases have been identified in hematopoietic and nonhematopoietic malignancies. Recently, we and others identified a single recurrent somatic activating mutation (JAK2V617F) in the Janus kinase 2 (JAK2) tyrosine kinase in the myeloproliferative disorders (MPDs) polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. We used direct sequence analysis to determine if the JAK2V617F mutation was present in acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML)/atypical chronic myelogenous leukemia (aCML), myelodysplastic syndrome (MDS), B-lineage acute lymphoblastic leukemia (ALL), T-cell ALL, and chronic lymphocytic leukemia (CLL). Analysis of 222 patients with AML identified JAK2V617F mutations in 4 patients with AML, 3 of whom had a preceding MPD. JAK2V617F mutations were identified in 9 (7.8%) of 116 CMML/a CML samples, and in 2 (4.2%) of 48 MDS samples. We did not identify the JAK2V617F disease allele in B-lineage ALL (n = 83), T-cell ALL (n = 93), or CLL (n = 45). These data indicate that the JAK2V617F allele is present in acute and chronic myeloid malignancies but not in lymphoid malignancies.

NGUYEN-KHAC F., DAVI F., RECEVEUR A., MALOUM K., MOREL V., LE GARFF-TAVERNIER M., ONG J., BERGER R., LEBLOND V., MERLE-BERAL H.

Burkitt-type acute leukemia in a patient with B-prolymphocytic leukemia: evidence for a common origin.

Cancer Genet. Cytogenet., 159 (1), 74-78, 2005

(Services cités : [E 0210](#))

Burkitt-type acute leukemia cells were present in the bone marrow of a patient with B-prolymphocytic leukemia diagnosed from peripheral blood cell morphology. Immunophenotype analysis confirmed morphological patterns. Cytogenetic and fluorescence in situ hybridization (FISH) analysis showed an identical t(8;22)(q24;q21) with MYC locus rearrangement in blood and bone marrow cells, with additional chromosome abnormalities in the bone marrow. In addition, the loss of one copy of the TP53 gene and identical IGH DNA clonal rearrangements were shown with FISH and polymerase chain reaction analysis respectively in the two types of leukemic cells. These data indicated the common origin of the two coexisting leukemias and are the first example of such occurrence in a leukemic patient.

STEFFANN J., FEYEREISEN E., KERBRAT V., ROMANA S., FRYDMAN N.

Prenatal and preimplantation genetic diagnosis : new practices.

M S-Méd. Sci., 21 (11), 987-992, 2005

(Services cités : E 0210, Génétique Médicale Pédiatrique, U393)

Preimplantation genetic diagnosis (PGD) purpose is to assess the genetic status of 3 day-old embryos. PGD offers thus to couples << at-risk >> of a genetic disorder an earlier option to prenatal diagnosis (PND). At the beginning, PGD's indications, patients and law were very closed to PND, but PGD specificities are gradually raising. Particularly, indications vary considerably in countries where the absence of law authorizes all the practices. Some of these applications are moreover raising serious ethical issues. Even in France, where this activity is particularly supervised, the recent modification to the law marks this evolution. double dagger.

2004

ARNAUD M., MZALI R., GESBERT F., CROUIN C., GUENZI C., VERMOT-DESROCHES C., WIJDENES J., COURTOIS G., BERNARD O., BERTOGLIO J.

Interaction of the tyrosine phosphatase SHP-2 with Gab2 regulates Rho-dependent activation of the c-fos serum response element by interleukin-2.

Biochem. J., 382 (Pt 2), 545-546, 2004

(Services cités : E 0210)

Gab2 (Grb2-associated binder-2), a member of the IRS (insulin receptor substrate)/Gab family of adapter proteins, undergoes tyrosine phosphorylation in response to cytokine or growth factor stimulation and serves as a docking platform for many signal transduction effectors, including the tyrosine phosphatase SHP-2 [SH2 (Src homology 2)-domain-containing tyrosine phosphatase]. Here, we report that, following IL-2 (interleukin-2) stimulation of human T lymphocytes, SHP-2 binds tyrosine residues 614 and 643 of human Gab2 through its N- and C-terminal SH2 domains respectively. However, the sole mutation of Tyr-614 into phenylalanine is sufficient to prevent Gab2 from recruiting SHP-2. Expression of the Gab2 Tyr-614-->Phe (Y614F) mutant, defective in SHP-2 association, prevents ERK (extracellular-signal-regulated kinase) activation and expression of a luciferase reporter plasmid driven by the c-fos SRE (serum response element), indicating that interaction of SHP-2 with Gab2 is required for ERK activation in response to IL-2. Further investigation of IL-2-dependent induction of SRE showed that expression of a constitutively active mutant of the RhoA GTPase synergizes with IL-2 for SRE-driven transcription, whereas a dominant-negative mutant reduces the IL-2 response. Thus, in response to IL-2, full induction of the SRE requires ERK-dependent as well as Rho-dependent signals that target the Ets-box and the CArG-box respectively. We also report that the synergy between Gab2/SHP-2 and RhoA for IL-2-dependent CArG-box-driven transcription depends upon MEK (mitogen-activated protein kinase/ERK kinase) activation, and is likely to involve regulation of the serum response factor co-activator MAL. Our studies thus provide new insights into the role of Gab2 and SHP-2 in IL-2 signal transduction.

ASNAFI V., BELDJORD K., LIBURA M., VILLARESE P., MILLIEN C., BALLERINI P., KUHLEIN E., LAFAGE-POCHITALOFF M., DELABESSE E., BERNARD O., MACINTYRE E.

Age-related phenotypic and oncogenic differences in T-cell acute lymphoblastic leukemias may reflect thymic atrophy.

Blood, 104 (13), 4173-4180, 2004

(Services cités : E 0210, Laboratoire d'Hématologie)

Postnatal thymic involution occurs progressively throughout the first 3 decades of life. It predominantly affects T-cell receptor (TCR) alphabeta-lineage precursors, with a consequent proportional increase in multipotent thymic precursors. We show that T-acute lymphoblastic leukemias (T-ALLs) demonstrate a similar shift with age from predominantly TCR expressing to an immature (IM0/delta/gamma) stage of maturation arrest. Half demonstrate HOX11, HOX11L2, SIL-TAL1, or CALM-AF10 deregulation, with each being associated with a specific, age-independent stage of maturation arrest. HOX11 and SIL-TAL represent alphabeta-lineage oncogenes, whereas HOX11L2 expression identifies an intermediate alphabeta/gammadelta-lineage stage of maturation arrest. In keeping with preferential alphabeta-lineage involution, the incidence of SIL-TAL1 and HOX11L2 deregulation decreased with age. In contrast, HOX11 deregulation became more frequent, suggesting longer latency. TAL1/LMO1 deregulation is more frequent in alphabeta-lineage T-ALL, when it is predominantly due to SIL-TAL1 rearrangements in children but to currently unknown mechanisms in adolescents and adults. LMO2 was more frequently coexpressed with LYL1, predominantly in IM0/delta/gamma adult cases, than with TAL1. These age-related changes in phenotype and oncogenic pathways probably reflect progressive changes in the thymic population at risk of malignant transformation.

ASNAFI V., BELDJORD K., GARAND R., MILLIEN C., BAHLOUL M., LETUTOUR P., DOUAY L., VALENSI F., MACINTYRE E.

IgH DJ rearrangements within T-ALL correlate with cCD79a expression, an immature/TCRgammadelta phenotype and absence of IL7Ralpha/CD127 expression.

Leukemia, 18 (12), 1997-2001, 2004

(Services cités : E 0210, Laboratoire d'Hématologie)

cCD79a and IgH VDJ/DJ rearrangements are considered to be relatively specific for B lymphoid precursors. We looked for both in cCD3+, CD7+, CD19- T-ALLs classified by TCR status into alphabeta or gammadelta/immature (IM) lineages, with individualization of HOX11L2+ T-ALLs since they represent an intermediate alphabeta/gammadelta category. cCD79a was expressed at low levels in 47% of T-ALL and was most frequent in IMgamma T-ALLs. IgH rearrangements were common in gammadelta/IM (45%) and HOX11L2+ (35%) T-ALLs compared to HOX11L2-negative cases (3%; P<0.001). CD127 (IL7Ralpha) expression was also more common in the gammadelta/IM lineage but its expression was virtually mutually exclusive of IgH rearrangement. Low-level cCD79a expression alone should therefore not be interpreted as evidence of B lineage affiliation in immature leukemias. gammadelta/IM lineage T-ALLs potentially include two distinct categories: predominantly IgH+, cCD79a+, CD127- cases which retain gammadelta and B lymphoid potential and IgH-, cCD79a-, CD127+ cases with restricted T lineage potential. *Leukemia* (2004) 18, 1997-2001. doi:10.1038/sj.leu.2403531 Published online 14 October 2004.

BERGER R.

Acquired trisomy 2 is not systematically associated with tumors.

Cancer Genet. Cytogenet., 153 (1), 86-87, 2004

(Services cités : E 0210)

BUSSON M., ROMANA S., KHAC F.N., BERNARD O., BERGER R.

Cryptic translocations involving chromosome 20 in polycythemia vera.

Ann. Génét., 47 (4), 365-371, 2004

(Services cités : E 0210)

A systematic cytogenetic study was performed in 49 patients with polycythemia vera (PV) in order to investigate the occurrence of subtelomeric rearrangements of chromosome 20, the most frequently rearranged chromosome in this myeloproliferative disorder. Partial deletion of the long arm of chromosome 20 was observed in two patients and two cryptic translocations, t(1;20)(p36;q13) and t(18;20)(p11;q13) in two others, all previously treated. The localization of the breakpoints of the translocated 20 chromosomes was different in the two translocations, as shown by fluorescence in situ hybridization (FISH) to metaphase chromosomes using BAC clones. Although infrequent (2/49), cryptic translocations of chromosome 20 deserve to be detected as preliminary to identification of molecular defects in PV.

DELLA VALLE V., GUGLIELMI L., BUSSON M., ZWARTHOF E.C., BERGER R., BERNARD O.A.

Expression of the MN1-TEL fusion protein in the human UCSD/AML1 leukemic cell line.

Leukemia, 18 (9), 1558-1560, 2004

(Services cités : E 0210, IRNEM)

RECEVEUR A., ONG J., MERLIN L., AZGUI Z., MERLE-BERAL H., BERGER R., NGUYEN-KHAC F.

Trisomy 4 associated with double minute chromosomes and MYC amplification in acute myeloblastic leukemia.

Ann. Génét., 47 (4), 423-427, 2004

(Services cités : E 0210, Laboratoire d'Hématologie)

A case of de novo acute myeloblastic leukemia (AML) M2, with trisomy 4 and double minute (dmin) chromosomes is reported. Amplification of the MYC gene ascertained by FISH was associated with dmin. A review of the literature of trisomy 4-dmin-associated AML shows that this entity preferentially occurs in elderly women and is not always associated with previously identified exposition to mutagens.

SU X.Y., BUSSON M., DELLA VALLE V., BALLERINI P., DASTUGUE N., TALMANT P., FERRANDO A.A., BAUDRY-BLUTEAU D., ROMANA S., BERGER R., BERNARD O.A.

Various types of rearrangements target TLX3 locus in T-cell acute lymphoblastic leukemia.

Genes Chromosome Cancer, 41 (3), 243-249, 2004

(Services cités : E 0210)

Most chromosomal translocations observed in T-cell acute lymphoblastic leukemia (T-ALL) often produce transcriptional activation of transcription factor oncogenes. Ectopic expression of the TLX3 (also known as HOX11L2) gene has been shown to be associated with a cryptic t(5;14)(q35;q32) translocation specific for a subtype of T-ALL. Here we report several examples of variant and alternative translocations resulting in expression of TLX3 in T-ALL, and we describe three of these translocations in detail. In particular, the CDK6 gene was rearranged in two t(5;7)(q35;q21) translocations. In two additional instances, fusion of the BCL11B (also known as CTIP2) and RANBP17/TLX3 loci were shown to result from subtle genomic insertion/deletion within these loci. This study further underscores that TLX3 expression in T-ALL is strongly associated with the presence of genomic rearrangements. Copyright 2004 Wiley-Liss, Inc.

2003

ASNAFI V., RADFORD-WEISS I., DASTUGUE N., BAYLE C., LEBOEUF D., CHARRIN C., GARAND R., LAFAGE-POCHITALOFF M., DELABESSE E., BUZYN A., TROUSSARD X., MACINTYRE E.

CALM-AF10 is a common fusion transcript in T-ALL and is specific to the TCRgammadelta lineage._____

Blood, 102 (3), 1000-1006, 2003

(Services cités : Laboratoire d'Hématologie, E 0210)

The t(10;11)(p13-14;q14-21) associated with CALM-AF10 is considered to be rare and associated with a variety of acute lymphoid and myeloid leukemias. Twelve (9%) of 131 unselected T-cell acute lymphoid leukemias (T-ALLs) expressed CALM-AF10 by reverse transcription-polymerase chain reaction or fluorescence in situ hybridization (or both), including 8% of children and 10% of adults, of whom only half demonstrated a t(10;11) by classical cytogenetics. CALM-AF10 was not found in T-cell-receptor alphabeta (TCRalphabeta) lineage T-ALLs, as defined by expression of TCRalphabeta, cytoplasmic TCRbeta, or TCRbetaVDJ rearrangement in immature cytoplasmic TCRbeta- cases, compared with 19% of TCRgammadelta T-ALLs and 33% of immature delta/gamma T-ALLs. The latter differed from their CALM-AF10- immature counterparts by a CD5+/CD2-phenotype, as found in TCRgammadelta but not TCRalphabeta T-ALLs and in their TCRgamma and TCRdelta configurations, altogether suggesting that CALM-AF10+ immature delta/gamma T-ALLs are TCRgammadelta precursors and that, within T-ALL, CALM-AF10 is specific for this lineage. Nine of 12 immature CALM-AF10 T-ALLs demonstrated 3' fusion transcripts, whereas 6 of 7 TCRgammadelta T-ALLs demonstrated 5' fusion transcripts. The latter retain the AF10 extended LAP/PHD domain necessary for homo-oligomerization. All 8 patients with CALM-AF10+TCRgammadelta T-ALLs are alive, compared with only 3 of 12 with immature CALM-AF10+ T-ALLs. Six CALM-AF10+ non-T acute leukemias all expressed CD7 and demonstrated T-restricted TCRdelta rearrangements, suggesting that they may also be related to the TCRgammadelta lineage. CALM-AF10 is therefore the most common fusion protein in T-ALL. It requires molecular and immunophenotypic characterization for appropriate prognostic evaluation and should be included in diagnostic screening panels of T-ALL and immature acute leukemias. Analysis of immature CALM-AF10+ leukemias will also facilitate analysis of the early stages of development of the TCRgammadelta lineage.

BALLERINI P., BLAISE A., MERCHER T., PELLEGRINO B., PEROT C., VAN DEN AKKER J., GATBOIS E., ADAM M., DOUAY L., BERGER R., BERNARD O., LANDMAN-PARKER J.

A novel real-time RT-PCR assay for quantification of OTT-MAL fusion transcript reliable for diagnosis of t(1;22) and minimal residual disease (MRD) detection.

Leukemia, 17 (6), 1193-1196, 2003

(Services cités : E 0210)

Leukemia (2003) 17, 1193-1196. doi:10.1038/sj.leu.2402914

BERGER R., CONIAT M.B.

Double trisomy 8 and 21 in acute myelocytic leukemias, one with rearrangement of the RUNX1 gene.

Cancer Genet. Cytogenet., 142 (2), 158-161, 2003

(Services cités : E 0210)

Fluorescence in situ hybridization analysis was carried out in five patients with acute

myeloblastic leukemia of various French-American-British subtypes and with double trisomy of chromosomes 8 and 21. PML-RARA fusion was detected with appropriate molecular probes in one patient with acute promyelocytic leukemia without t(15;17). Two PAC probes covering the 5' and 3' part of the RUNX1 gene were used in the four other patients. While three copies were present in three patients, as expected from conventional karyotype analysis, only two hybridization signals were present in the fifth patient. This was due to the apparent loss of the 3' part of RUNX1. Since chromosome number abnormalities may be associated with submicroscopic gene rearrangements, it should be important to search for them for a better understanding of mechanisms of leukemogenesis, and to understand the prognostic heterogeneity in leukemic patients with aneusomies without apparent chromosome structure rearrangements.

BERGER R., DASTUGUE N., BUSSON M., VAN DEN AKKER J., PEROT C., BALLERINI P., HAGEMEIJER A., MICHAUX L., CHARRIN C., PAGES M.P., MUGNERET F., ANDRIEUX J., TALMANT P., HELIAS C., MAUVIEUX L., LAFAGE-POCHITALOFF M., MOZZICONACCI M.J., CORNILLET-LEFEBVRE P., RADFORD I., ASNAFI V., BILHOU-NABERA C., NGUYEN-KHAC F., LEONARD C., SPELEMAN F., POPPE B., BASTARD C., TAVIAUX S., QUILICHINI B., HERENS C., GREGOIRE M.J., CAVE H., BERNARD O.A.

t(5;14)/HOX11L2-positive T-cell acute lymphoblastic leukemia. A collaborative study of the Groupe Francais de Cytogenetique Hematologique (GFCH).

Leukemia, 17 (9), 1851-1857, 2003

(Services cités : E 0210)

To accurately estimate the incidence of HOX11L2 expression, and determine the associated cytogenetic features, in T-cell acute lymphoblastic leukemia (T-ALL), the Groupe Francais de Cytogenetique Hematologique (GFCH) carried out a retrospective study of both childhood and adult patients. In total, 364 patients were included (211 children ≤ 15 years and 153 adults), and 67 (18.5%) [47 children (22.4%) and 20 adults (13.1%)] were shown to either harbor the t(5;14)q35;q32 translocation or express the HOX11L2 gene or both. Most of the common hematological parameters did not show significant differences within positive and negative populations, whereas the incidence of CD1a+/CD10+ and cytoplasmic CD3+ patients was significantly higher in positive than in negative children. Out of the 63 positive patients investigated by conventional cytogenetics, 32 exhibited normal karyotype, whereas the others 31 showed clonal chromosome abnormalities, which did not include classical T-ALL specific translocations. Involvement of the RANBP17/HOX11L2 locus was ascertained by fluorescence in situ hybridization in six variant or alternative (three-way translocation or cytogenetic partner other than 14q32) translocations out of the 223 patients. Our results also show that HOX11L2 expression essentially occurs as a result of a 5q35 rearrangement, but is not associated with another identified T-ALL specific recurrent genetic abnormality, such as SIL-TAL fusion or HOX11 expression. *Leukemia* (2003) 17, 1851-1857. doi:10.1038/sj.leu.2403061

BERGER R.

Evolution of a double agent: cytogenetics in hematology.

Pathol. Biol., 51 (6), 305-306, 2003

(Services cités : E 0210)

GENEVIEVE D., CORMIER-DAIRE V., SANLAVILLE D., FAIVRE L., GOSSET P., ALLART L., PICQ M., MUNNICH A., ROMANA S., de BLOIS M., VEKEMANS M.

Mild phenotype in a 15-year-old boy with Pallister-Killian syndrome.

Amer. J. Med. Genet., 116 (1), 90-93, 2003

(Services cités : E 0210, Génétique Médicale Pédiatrique)

Pallister-Killian syndrome is a rare disorder characterized by multiple congenital anomalies, coarse face, pigmentary skin changes, seizures, severe mental retardation, and the presence of an extra metacentric chromosome i(12p) confined to skin fibroblasts only. Here, we report on an unusual case of i(12p) in a 15-year-old boy presenting with mild mental retardation, minor facial features (long face, prognathism, short neck), normal weight, length, and OFC parameters as well as hyperpigmented streaks. The boy attended normal school until the age of 14 years. Because of hyperpigmented stripes, chromosome analysis was performed on skin fibroblasts. This study showed that 37% of the cells had an additional isochromosome for the short arm of chromosome 12. This observation illustrates the phenotypic variability of i(12p) and emphasizes the importance of skin fibroblasts chromosome analysis in patients with pigmentary skin changes.

MENTION J.J., BEN AHMED M., BEGUE B., BARBE U., VERKARRE V., ASNAFI V., COLOMBEL J.F., CUGNENC P.H., RUEMMELE F.M., MCINTYRE E., BROUSSE N., CELLIER C., CERF-BENSUSSAN N.

Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease.

Gastroenterology, 125 (3), 730-745, 2003

(Services cités : E 0212, E 0210)

BACKGROUND & AIMS: The mechanism of intraepithelial lymphocyte hyperplasia, a hallmark of celiac disease, is unknown. We have investigated the role of epithelium-derived interleukin (IL)-15 in the alterations of epithelial homeostasis in refractory celiac sprue, a privileged situation to study the first step of lymphoid transformation and the contribution of intraepithelial lymphocytes to villous atrophy in celiac disease. **METHODS:** IL-15 expression was assessed in biopsy specimens and isolated enterocytes by combining immunohistochemistry, flow cytometry, and real-time quantitative polymerase chain reaction. The ability of IL-15 to induce growth and survival of clonal intraepithelial lymphocytes lacking surface CD3 and to induce their cytotoxicity and secretion of interferon gamma was tested using soluble IL-15 and coculture in the presence of epithelial cell lines expressing membrane IL-15. **RESULTS:** IL-15 was massively overexpressed not only in lamina propria but also in the intestinal epithelium of patients with active celiac disease and refractory celiac sprue. IL-15 was not secreted but delivered at the surface of enterocytes. IL-15 specifically induced the expansion and survival of the clonal abnormal intraepithelial lymphocytes that characterize refractory celiac sprue and triggered their secretion of interferon gamma and their cytotoxicity against intestinal epithelial cells. Comparable activating signals could be delivered by IL-15 expressed at the membrane of the T84 enterocyte cell line. **CONCLUSIONS:** These data provide strong evidence that uncontrolled overexpression of IL-15 in refractory celiac sprue perpetuates epithelial damage and promotes the emergence of T-cell clonal proliferations. Blocking IL-15 might prove useful to treat this severe complication of celiac disease.

NGUYEN-KHAC F., BERNARD O.A.

Chromosomal translocations in human malignant hematopoiesis. Structural and functional consequences.

Pathol. Biol., 51 (6), 382-389, 2003

(Services cités : E 0210)

The improvement of molecular biology techniques and human genome mapping and sequencing boosted the molecular analysis of chromosomal abnormalities observed in human hematological malignancies. The characterization of structural abnormalities (translocation, deletion) has proven particularly seminal. A better understanding of the pathology itself and of its generation arose from the identification of the genes involved in the chromosomal translocations of human leukemia. This work summarizes some of the present knowledge regarding human leukemogenesis.

VAN DONGEN J.J., LANGERAK A.W., BRUGGEMANN M., EVANS P.A., HUMMEL M., LAVENDER F.L., DELABESSE E., DAVI F., SCHUURING E., GARCIA-SANZ R., VAN KRIEKEN J.H., DROESE J., GONZALEZ D., BASTARD C., WHITE H.E., SPAARGAREN M., GONZALEZ M., PARREIRA A., SMITH J.L., MORGAN G.J., KNEBA M., MACINTYRE E.A.

Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936.

Leukemia, 17 (12), 2257-2317, 2003

(Services cités : Laboratoire d'Hématologie, E 0210)

In a European BIOMED-2 collaborative study, multiplex PCR assays have successfully been developed and standardized for the detection of clonally rearranged immunoglobulin (Ig) and T-cell receptor (TCR) genes and the chromosome aberrations t(11;14) and t(14;18). This has resulted in 107 different primers in only 18 multiplex PCR tubes: three VH-JH, two DH-JH, two Ig kappa (IGK), one Ig lambda (IGL), three TCR beta (TCRB), two TCR gamma (TCRG), one TCR delta (TCRD), three BCL1-Ig heavy chain (IGH), and one BCL2-IGH. The PCR products of Ig/TCR genes can be analyzed for clonality assessment by heteroduplex analysis or GeneScanning. The detection rate of clonal rearrangements using the BIOMED-2 primer sets is unprecedentedly high. This is mainly based on the complementarity of the various BIOMED-2 tubes. In particular, combined application of IGH (VH-JH and DH-JH) and IGK tubes can detect virtually all clonal B-cell proliferations, even in B-cell malignancies with high levels of somatic mutations. The contribution of IGL gene rearrangements seems limited. Combined usage of the TCRB and TCRG tubes detects virtually all clonal T-cell populations, whereas the TCRD tube has added value in case of TCRgammadelta(+) T-cell proliferations. The BIOMED-2 multiplex tubes can now be used for diagnostic clonality studies as well as for the identification of PCR targets suitable for the detection of minimal residual disease.

2002

BALLERINI P., BLAISE A., BUSSON-LE CONIAT M., SU X.Y., ZUCMAN-ROSSI J., ADAM M., VAN DEN AKKER J., PEROT C., PELLEGRINO B., LANDMAN-PARKER J., DOUAY L., BERGER R., BERNARD O.A.

HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis.

Blood, 100 (3), 991-997, 2002

(Services cités : EMI 0210)

The most frequent oncogenic activation events characterized in childhood T acute lymphoblastic leukemia (T-ALL) result in the transcriptional activation of genes coding for transcription factors. The main genes are TAL1/SCL, a member of the basic region helix-loop-helix gene family, and HOX11L2, a member of the homeobox-containing protein family. To gain insight into the

pathogenesis of this type of hematologic malignancy, we analyzed 28 T-ALL samples. SIL-TAL1/SCL fusion was detected in 6 patients; expression of HOX11L2 was observed in 6 patients and of HOX11 in 3 patients. With one exception, these activations did not occur simultaneously in the same patients, and they allowed the subclassification of 50% of the patients. SIL-TAL1 fusion was detected in association with HOX11 expression in one patient and with a t(8;14) (q24;q11) in another. High expression of LYL1, LMO2, or TAL1 was observed mainly in samples negative for HOX11L2 expression. HOX11L1 and HOX11 expression were observed in one instance each, in the absence of detectable chromosomal abnormality of their respective loci, on chromosomes 2 and 10, respectively. HOX11L2 expression was associated with a chromosome 5q abnormality, the location of the HOX11L2 locus in each case tested. Finally, our data show that HOX11L2 expression was a suitable marker for minimal residual disease follow-up and was significantly associated with relapse (P =.02). (Blood. 2002;100:991-997)

BUSSON-LE CONIAT M., BOUCHER N., BLANCHE H., THOMAS G., BERGER R.

Chromosome studies of in vitro senescent lymphocytes: nonrandom trisomy 2.

Ann. Génét. Paris, 45 (4), 193-196, 2002

(Services cités : EMI 0210)

Chromosome studies were carried out in long-term (142 and 184 d) human lymphocyte in vitro cultures in order to investigate the cytogenetic status of aging lymphocytes. The female donors were subdivided into three subgroups according to their age: 20-40 year-old (three individuals), 70-90 year-old (five persons), and centenarians (three persons). Besides some aneuploidy and structural abnormalities, telomere fusions were detected in all donor cells, and associations of acrocentric chromosomes were found in six persons in the three age-groups. Clonal trisomy 2 was present in three individuals (two from the 70-90 year-group and one centenarian with a clone +2, +8). While telomeric fusions and acrocentric associations seem to be more related to in vitro aging, trisomy 2 also appears dependent on the age of the cell donors.