PL, Holyoake AJ, Fichelson S, et al. A mutation of human cytochrome c enhances the intrinsic apoptotic pathway but causes only thrombocytopenia. Nat Genet 2008;40: 387-9.
17. Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature 2008;451:335-9.
18. Buonamici S, Li D, Chi Y, Zhao R, Wang X, Brace L, et al. EVI1 induces myelodysplastic syndrome in mice. J Clin Invest 2004;114:713-9.
19. Watanabe-Okochi N, Kitaura J, Ono R, Harada H, Harada Y, Komeno Y, et al. AML1 mutations induced MDS and MDS/AML in a mouse BMT model. Blood 2008;111:4297-308.
20. Omidvar N, Kogan S, Beurlet S, le Pogam C, Janin A, West R, et al. BCL-2 and mutant NRAS interact physically and functionally in a mouse model of progressive myelodysplasia. Cancer Res 2007;67:11657-67.
21. Rassool FV, Gaymes TJ, Omidvar N, Brady N, Beurlet S, Pla M, et al. Reactive oxygen species, DNA damage, and error-prone repair: a model for genomic instability with progression in myeloid leukaemia. Cancer Res 2007;67: 8762-71.
22. Grisendi S, Bernardi R, Rossi M, Cheng K, Khandler L, Manova K, et al. Role of nucleophosmin in embryonic development and tumorigenesis. Nature 2005;437:147-53.
23. Lin YW, Slape C, Zhang Z, Aplan PD. NUP98-HOXD13 transgenic mice develop a highly penetrant, severe myelodysplastic syndrome that progresses to acute leukemia. Blood 2005;106:287-95.
24. Choi CW, Chung YJ, Slape C, Aplan PD. Impaired differentiation and apoptosis of hematopoietic precursors in a mouse model of myelodysplastic syndrome. Haematologica 2008;93:1394-97.
25. Slape C, Liu LY, Beachy S, Aplan PD. Leukemic transformation in mice expressing a NUP98-HOXD13 transgene is accompanied by spontaneous mutations in Nras, Kras, and Cbl. Blood 2008;[Epub ahead of print].

## The pathogenesis of classical Hodgkin's lymphoma: what can we learn from analyses of genomic alterations in Hodgkin and Reed-Sternberg cells?

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Classical Hodgkin's lymphoma (HL) is one of the most common human malignant lymphomas. Due to its characteristic histopathological, molecular and clinical features HL can be distinguished from all other lymphomas, the so-called non-Hodgkin's lymphomas. Two findings, in particular, make HL a unique malignant hematopoietic disease: first, the malignant mononucleated Hodgkin- and the multinucleated Reed-Sternberg cells (in the following termed Hodgkin/Reed-Sternberg [HRS] cells) represent only a small fraction of cells in the affected lymph nodes and are embedded in an infiltrate of reactive hematopoietic cells. Among others, these reactive cells are composed of B- and T-lymphocytes, eosinophils and plasma cells, and they are usually considered to be non-malignant. Second, the malignant HRS cells do not display a phenotype that can be assigned to a defined hematopoietic cell type.[1] The origin of HRS cells has, therefore, long been a matter of debate. With the description of clonally rearranged immunoglobulin (Ig) genes with a high frequency of somatic mutations it became clear that HRS cells are in most cases derived from germinal center (GC) or post-GC B cells.[2] It is of particular interest to note that, despite their B-cell origin, HRS cells have, in most cases, lost the B-cell-specific gene expression program. This is in contrast to most other B-cell-derived lymphomas, the gene expression pattern of which usually reflects the differentiation stage of their respective cell of origin. Furthermore, it is unclear how such cells survive, as GC B cells with defective Ig production usually undergo apoptotic cell death.

Clinically, HL is curable at the early stages in the vast majority of cases. However, the treatment of patients with advanced disease stages or insufficient response to the initial standard treatment options is challenging.[3] Furthermore, treatment succes is linked to considerable long-term toxicity, including the risk of treatment-related secondary malignancies. It is, therefore, imperative to develop less toxic therapeutic strategies, in particular non-genotoxic treatments based on, for example, inhibition of signaling pathways required for growth and survival of the tumor cells or enforcement of a cellular differentiation program leading to growth arrest and apoptosis. Knowledge of the key genomic and molecular defects of HRS cells is fundamental for the development of such treatment strategies.

The rareness of the tumor cells in the affected lymph nodes has been and is still a major obstacle to the identification of molecular and genomic defects in HRS cells and to the possibility of performing functional analyses with primary HRS cells. Although the purification of viable primary HRS cells from patients' lymph nodes has been described,[4] functional studies with these cells turned out to be nearly impossible because of the lack of suitable sample material and the insufficient number of cells. The current knowledge of molecular defects in HL is, therefore, based almost entirely on work with cell lines, albeit well-characterized, the analysis of primary HRS cells by immunohistochemistry and the analysis of micromanipulated HRS cells, as performed in the study by Hartmann *et al.* published in this issue of the journal.[5]

### Deregulated signaling pathways in classical Hodgkin's lymphoma

Despite great efforts and advances in the last years, the pathogenesis of HL has not been clarified. In particular, although at the genomic level several recurrent alterations have been described, no unifying genomic defect specific to the malignant HRS cells has yet been identified. However, at the molecular level, a number of characteristic molecular defects have been demonstrated. These include activation of the transcription factors nuclear factor kappa B (NF-κB), activator protein 1 (AP-1), members of the STAT signaling pathway and dereg-

ulated NOTCH1 signaling.[1] The deregulation of these signaling pathways is found in the vast majority or even all HRS cells of nearly all HL cases analyzed, pointing to the central relevance of these pathways for HRS cell survival, growth and regulation of HRS cell-specific gene expression. The NF-κB and AP-1 transcription factors are thought to synergistically regulate not only genes involved in apoptosis protection and proliferation but also the abundant number of cytokines and chemokines produced by the HRS cells.[1] The AP-1 and NOTCH1 signaling pathways have been shown to be involved in the loss of the B-cell phenotype,[6,7] characteristic of the HRS cells. The simultaneous constitutive activation of all these signaling pathways is unique to HL and reveals that HRS cells require strong cell-autonomous signals ensuring growth and apoptosis protection.

### The delineation of genomic alterations in HRS cells

A growing number of studies are aimed at identifying genomic alterations or translocations specific to HRS cells of HL. Due to the rareness of the tumor cells and the resulting difficulties in obtaining sufficient numbers of metaphase tumor cells for cytogenetic analyses, data on genomic alterations of HRS cells are still limited. The available data point to an unusual complex karyotype of the HRS cells with ongoing rearrangment activity.[8-12] Up to now, no single unifying genomic defect has been described for HRS cells. This is in contrast to non-Hodgkin's lymphomas which frequently harbor genetic lesions, especially translocations, that, albeit not absolutely specific, are closely associated with a distinct entity. Nevertheless, a number of recurrent defects have been identified, including gains or amplifications of the gene loci for *JAK2*, *FGFR3*, *REL* and *ID2*.[8,13] In particular, genomic gains in chromosomal arms 2p and 9p were reported as recurrent alterations in primary HRS cells.[8] Fluorescence *in situ* hybridization analyses of these regions delineated copy number increases of the gene locus for the NF-κB transcription factor *REL* and the *JAK2* locus in a significant number of HL cases. These findings are in line with the presumed function of deregulated activity of the NF-κB and the JAK/STAT signaling pathways in HRS cells, supporting their pathogenetic role in these cells. More recently, chromosomal translocations involving the *IG* loci were found in approximately 20% of HL cases.[12] Their pathogenetic significance is unclear, since the assumed mechanism of such translocations is the juxtaposition of a pathogenetically relevant gene to an actively transcribed *IG* locus, whereas the *IG* locus is not active in HRS cells. However, it cannot be excluded that such genetic events contribute to the pathogenesis of HL at a given time point during lymphomagenesis. Alternatively, in such cases the deregulated expression of genes with a putative transforming capacity might be the result of positioning them in an altered chromatin context. In summary, analyses of genomic alterations in HRS cells have confirmed the significance of deregulated signaling pathways identified by molecular biology approaches. Furthermore, they have revealed additional defects, whose relevance for HRS cell biology has yet to be elucidated. However, to date a unifying genomic defect providing a concept for HRS cell biology has still not been identified.

### Questions to be answered in the future

The work by Hartmann *et al.*[5] represents a further step towards a detailed map of genetic lesions in HL. Their study confirms and extends previous reports of chromosomal imbalances in HRS cells, including increased copy numbers of the gene loci for *JAK2*, *RELB*, *STAT6*, *NOTCH1* and *JUNB*. The recurrent finding of chromosomal gains in these regions indicates that the pathogenesis of HL is associated with a selection pressure for the accumulation of such genetic lesions, and clearly supports the biological significance of these genes for HRS cells of HL. However, whereas alterations in these genes are found only in a certain proportion of HL specimens, the deregulated activity of the respective signaling pathways is a strikingly consistent feature of the vast majority or even all HL cases.[1] This indicates that genomic alterations are just one mechanism among others leading to the activation of disease-relevant signaling cascades in HRS cells. Furthermore, it remains unclear at what stage of the oncogenic process the genomic aberrations occur, i.e. whether a given increase in copy number is an early or late event during transformation. One possible approach to answer this question could be to analyze composite lymphomas, using the same method as that applied by Hartmann *et al.* to HL cases, as has been previously described for the study of chromosomal translocations and tumor suppressor gene mutations.[14] Such studies could help to delineate genomic alterations that are closely associated with the development of HL and, therefore, likely to be highly relevant for the pathogenesis of this disease. In addition, serial analysis of HL cases at diagnosis and relapse could shed light on genetic changes associated with treatment failure and disease progression.

The unique histopathological pattern of HL, with rare HRS cells embedded in an abundant mixture of inflammatory cells, has repeatedly stimulated speculations on a relationship between HRS cells and their cellular environment. Previous reports described chromosomal abnormalities in a fraction of morphologically normal cells isolated from HL lymph nodes with common aberrations in both cell populations.[15] In contrast, studies analyzing the mutations of the *IKBA* gene, amplifications of the *JAK2* locus or translocations involving the *BCL3* gene found no evidence for the respective genetic changes in bystander cells.[9,16,17] The technical advances seen in the last years would enable this issue to be addressed in more detail and with much greater reliability, allowing definite clarification.

Technical advances are also making it possible to analyze genomic alterations associated with oncogenic transformation in ever greater detail, as reflected by Hartmann's work published in this issue of the journal. As a consequence, there is an increasing number of candidate genes that might contribute to the pathogenesis of HL. On the other hand, genomic alterations of genes playing a key role in HL might still be missed, as demonstrated by gains and amplifications of the *ID2*

locus, which were described by the same authors,[13] but were not identified in the current work. However, we are just beginning to understand the signaling networks and the cross-talk between AP-1, NF-κB and STAT transcription factors in HRS cells, and how they allow for synergistic activation of HRS cell-specific target genes, and the abundant number of highly expressed cytokines and chemokines.[1] Thus, it will be important in the future to develop high throughput approaches to define the biological role of identified candidate genes for HRS cell transformation and to determine their role within the complex network of deregulated proliferation and survival pathways. Furthermore, the analysis of genomic lesions by techniques such as array CGH will have to be complemented by studies aimed at identifying mutations in signaling molecules of lymphoma-relevant pathways, as already demonstrated for *SOCS1* and *IKBA* genes in HRS cells.[16,18] As exemplified by studies in diffuse large B-cell lymphoma and multiple myeloma, the genetic events underlying the activation of the canonical or alternative NF-κB pathways in B-cell malignancies can be very diverse, affecting numerous different components of the signaling cascade. Such studies will provide a more accurate molecular basis to answer the questions of which pathway and, moreover, which molecules in a given pathway are suitable targets for therapeutic interventions.

Another important issue of HL tumor biology concerns the complex and instable karyotype of the HRS cells. Although only limited data are available, and some of these data were generated with HRS cell-derived cell lines, it is remarkable that (i) chromosomes composed of multiple chromosomes of different origin are regularly found, (ii) some translocations are identified in only a subpopulation of cells, arguing for ongoing rearrangement activity in HRS cells, and (iii) small translocated chromosomal segments are identified in HRS cells.[8,10,11] In particular, such small segmental jumping translocations appear to be directly linked to gene amplification, as shown for *JAK2* or *FGFR3*.[8] All these findings point to an unusual genomic instability of HRS cells. It is not yet clear whether this instability is a cause or a consequence of HRS cell transformation. Is a viral infection involved in inducing such genomic instability, as has been proposed for human T-cell leukemia virus type-I infected cells in adult T-cell leukemia?[19] Furthermore, in pediatric HL cases, loss of expression of the ataxia telangiectasia mutated gene (*ATM*) has been reported,[20] and the constitutive activity of transcription factor NF-κB has been proposed to inhibit ATM expression in HRS cells.[21] The fact that ATM plays an important role in the response to DNA damage, and loss of ATM results in genomic instability,[22] suggests a functional link between ATM down-regulation and impaired DNA repair mechanisms in HL. In this context it is interesting to note that the genetic and functional status of the p53 pathway in HRS cells is not fully understood. Primary HRS cells frequently display high levels of p53 protein expression, an observation that is indicative of *p53* mutations. Whereas several HRS cell-derived cell lines have been shown to harbor small deletions in the *p53* gene,[23] *p53* mutations in primary HRS appear to be rare, at least based on sequencing studies that cover the hot spot regions for typical *p53* point mutations.[24] With respect to the molecular events that govern HL tumorigenesis as well as the rational development of treatment strategies, it will be critical to determine the mechanisms and consequences of the genomic instability in HRS cells in the future.

In summary, genomic studies of HRS cells have advanced our understanding of HL tumor biology by revealing a number of genetic defects with pathogenetic relevance, such as amplifications of genes encoding components of disease-associated signaling cascades and transcription factor networks. However, no recurrent HRS cell-characteristic genetic lesion has so far been identified. Thus, the genetic basis of HL has proven to be more complex than might have been expected or hoped. This has attenuated expectations that resolving the technical problems associated with the analysis of genomic imbalances in the rare population of HRS cells will reveal a specific genetic pattern for HL. The challenge for future work will be to integrate the results obtained by genetic and molecular biology approaches into a common pathogenetic concept, and to identify the unifying molecular defects of classical Hodgkin's lymphoma.

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## References

1. Re D, Küppers R, Diehl V. Molecular pathogenesis of Hodgkin's lymphoma. J Clin Oncol 2005;23:6379-86.
2. Küppers R, Rajewsky K. The origin of Hodgkin and Reed/Sternberg cells in Hodgkin's disease. Annu Rev Immunol 1998;16:471-93.
3. Diehl V, Engert A, Re D. New strategies for the treatment of advanced-stage Hodgkin's lymphoma. Hematol Oncol Clin North Am 2007;21:897-914.
4. Irsch J, Nitsch S, Hansmann ML, Rajewsky K, Tesch H, Diehl V, et al. Isolation of viable Hodgkin and Reed-Sternberg cells from Hodgkin disease tissues. Proc Natl Acad Sci USA 1998;95:10117-22.
5. Hartmann S, Martin-Subero JI, Gesk S, Hüsken J, Giefing M, Nagel I, et al. Detection of genomic imbalances in microdissected Hodgkin- and Reed-Sternberg cells of classical Hodgkin's lymphoma by array-based comparative genomic hybridization. Haematologica 2008;93:1318-26.
6. Mathas S, Janz M, Hummel F, Hummel M, Wollert-Wulf B, Lusatis S, et al. Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma. Nat Immunol 2006;7:207-15.
7. Jundt F, Acikgöz O, Kwon SH, Schwarzer R, Anagnostopoulos I, Wiesner B, et al. Aberrant expression of Notch1 interferes with the B-lymphoid phenotype of neoplastic B cells in classical Hodgkin lymphoma. Leukemia 2008;doi:10.1038/leu.2008.101.
8. Joos S, Granzow M, Holtgreve-Grez H, Siebert R, Harder L, Martin-Subero JI, et al. Hodgkin's lymphoma cell lines are characterized by frequent aberrations on chromosomes 2p and 9p including REL and JAK2. Int J Cancer 2003;103:489-95.
9. Joos S, Küpper M, Ohl S, von Bonin F, Mechtersheimer G, Bentz M, et al. Genomic imbalances including amplification of the tyrosine kinase gene JAK2 in CD30+ Hodgkin

cells. Cancer Res 2000;60:549-52.
10. Falzetti D, Crescenzi B, Matteuci C, Falini B, Martelli MF, Van Den Berghe H, et al. Genomic instability and recurrent breakpoints are main cytogenetic findings in Hodgkin's disease. Haematologica 1999;84:298-305.
11. Drexler HG. Recent results on the biology of Hodgkin and Reed-Sternberg cells. I. Biopsy material. Leuk Lymphoma 1992;8:283-313.
12. Martin-Subero JI, Klapper W, Sotnikova A, Callet-Bauchu E, Harder L, Bastard C, et al. Chromosomal breakpoints affecting immunoglobulin loci are recurrent in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma. Cancer Res 2006;66:10332-8.
13. Renne C, Martin-Subero JI, Eickernjäger M, Hansmann ML, Küppers R, Siebert R, et al. Aberrant expression of ID2, a suppressor of B-cell-specific gene expression, in Hodgkin's lymphoma. Am J Pathol 2006;169:655-64.
14. Schmitz R, Renne C, Rosenquist R, Tinguely M, Distler V, Menestrina F, et al. Insights into the multistep transformation process of lymphomas: IgH-associated translocations and tumor suppressor gene mutations in clonally related composite Hodgkin's and non-Hodgkin's lymphomas. Leukemia 2005;19:1452-8.
15. Jansen MP, Hopman AH, Bot FJ, Haesevoets A, Stevens-Kroef MJ, Arends JW, et al. Morphologically normal, CD30-negative B-lymphocytes with chromosome aberrations in classical Hodgkin's disease: the progenitor cell of the malignant clone? J Pathol 1999;189:527-32.
16. Emmerich F, Meiser M, Hummel M, Demel G, Foss HD, Jundt F, et al. Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells. Blood 1999; 94:3129-34.
17. Martin-Subero JI, Wlodarska I, Bastard C, Picquenot JM, Hoppner J, Giefing M, et al. Chromosomal rearrangements involving the BCL3 locus are recurrent in classical Hodgkin and peripheral T-cell lymphoma. Blood 2006;108:401-2.
18. Weniger MA, Melzner I, Menz CK, Wegener S, Bucur AJ, Dorsch K, et al. Mutations of the tumor suppressor gene SOCS-1 in classical Hodgkin lymphoma are frequent and associated with nuclear phospho-STAT5 accumulation. Oncogene 2006;25:2679-84.
19. Lemoine FJ, Marriott SJ. Genomic instability driven by the human T-cell leukemia virus type I (HTLV-I) oncoprotein, Tax. Oncogene 2002;21:7230-4.
20. Bose S, Starczynski J, Chukwuma M, Baumforth K, Wei W, Morgan S, et al. Down-regulation of ATM protein in HRS cells of nodular sclerosis Hodgkin's lymphoma in children occurs in the absence of ATM gene inactivation. J Pathol 2007;213:329-36.
21. Dutton A, Woodman CB, Chukwuma MB, Last JI, Wei W, Vockerodt M, et al. Bmi-1 is induced by the Epstein-Barr virus oncogene LMP1 and regulates the expression of viral target genes in Hodgkin lymphoma cells. Blood 2007;109: 2597-603.
22. Lavin MF, Birrell G, Chen P, Kozlov S, Scott S, Gueven N. ATM signaling and genomic stability in response to DNA damage. Mutat Res 2005;569:123-32.
23. Janz M, Stühmer T, Vassilev LT, Bargou RC. Pharmacologic activation of p53-dependent and p53-independent apoptotic pathways in Hodgkin/Reed-Sternberg cells. Leukemia 2007;21:772-9.
24. Montesinos-Rongen M, Roers A, Küppers R, Rajewsky K, Hansmann ML. Mutation of the p53 gene is not a typical feature of Hodgkin and Reed-Sternberg cells in Hodgkin's disease. Blood 1999;94:1755-60.

## Allogeneic transplantation in multiple myeloma

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Allogeneic hematopoietic stem cell transplantation has been performed for the treatment of multiple myeloma since the early 1980s. We performed our first myeloma transplant in April 1983.[1] The patient, a 46-year old woman, was diagnosed with monoclonal gammopathy of undetermined significance in 1974. The disease progressed to multiple myeloma and the patient required treatment with melphalan and prednisone in 1977. In 1982 the disease was resistant to chemotherapy and in early 1983 she received a bone marrow transplant from an HLA-identical brother. She engrafted without serious complications, and at the time of publication of her case report in 1986 she was in complete hematologic remission with no signs of disease. We thought that we had cured the first patient with multiple myeloma, but 4 years after the transplant she relapsed. She lived for another 6 years as a mixed chimera, but eventually died from the disease. Since then numerous allogeneic transplants have been performed throughout the world. The registry of the European Group of Blood and Marrow Transplantation (EBMT) has reports of more than 4000 transplants performed in European centers.

### Allogeneic transplantation using myeloablative conditioning

The idea of using high-dose myeloablative allogeneic transplantation in multiple myeloma has four rationales. First, the myeloablative chemotherapy and total body irradiation should eradicate the myeloma cells in the bone marrow. Second, reduction of host immunocompetent cells should allow engraftment of the allogeneic cells. Third, the graft should save the patient from the effect of ablation of normal host bone marrow cells. Fourth, the immunocompetent donor cells should help eradicate myeloma cells (through a graft-versus-myeloma [GVM], effect) that might persist despite the myeloablative therapy. Originally the most common myeloablative conditioning therapy was cyclophosphamide + total body irradiation (10-12 Gy), fractionated or unfractionated with lung shielding. However, many other myeloablative protocols have subsequently been developed.

Initially it appeared that all these four goals could be obtained in multiple myeloma. However, as in the case described above, it soon became apparent that this rarely happens. One problem is the high incidence of severe graft-versus-host disease (GVHD) and high transplant-related mortality, which reached 30-40%. Another problem was the significant relapse/progression rate.[2] Although the relapse/progression rate was shown to be lower with allogeneic transplantation than with autologous transplantation already in 1996 in an EBMT retrospective case-matched analysis of 378 patients, the overall survival was, at that time, inferior