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PROSPECT

Genetic Lesions and Perturbation of Chromatin Architecture: A Road to Cell Transformation

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Abstract Differential gene expression is a rigorously precise procedure that defines the developmental program of cells, tissues, organs, and of the entire organism. The correct execution of this program requires the participation of multiple and complex groups of regulators. In addition to transcription factors, which are key tools in ontogenesis by providing sequential switch of different genes, the structure of the chromatin is a dominant determinant leading to gene expression. Through the novel and insightful work of several investigators, it appears that the architecture of the chromatin spanning the genes can and does influence the efficiency of RNA transcription, and therefore of gene expression. Several new enzymatic complexes have been identified that reversibly modify the chromatin architecture by methylation, phosphorylation, and acetylation of the nucleosomal core proteins. These enzymes are crucial for the proper balance and maintenance of gene expression, and are often the target of mutations and alterations in human cancer. Here, we review briefly the current models proposing how some of these enzymes normally modify the chromatin structure and how their functional disruption leads to inappropriate gene expression and cell transformation. J. Cell. Biochem. 82: 310–325, 2001. © 2001 Wiley-Liss, Inc.

The dynamic structure of the chromatin and the ability to change its organization in response to environmental stimuli is the foundation of the ordered life of an eukaryotic cell. Twenty-five years ago, it was first proposed that the eukaryotic chromatin be structured in geometric repetitive units called nucleosomes [Kornberg, 1974]. In recent years, it has become clear that the nucleosomes not only provide structural support for ordered packing of the chromosomal DNA, but are also critical sites that control gene activation and repression, long-range locus activity and transcription, and probably DNA replication, DNA repair, and chromosomal recombination [Kornberg and Lorch, 1999]. At the center of the periodically assembled nucleosomal units are the highly conserved histone proteins, H2A, H2B, H3, and H4, that form the octameric core around which the DNA is folded. The assembly of DNA in nucleosomal units is a fluid structure that responds to the needs of the cell by remodeling regions that are actively involved in gene transcription and DNA replication. Large enzymatic complexes that regulate histone acetylation, methylation, and phosphorylation control chromatin remodeling. They modify N-terminus lysine residues of the histones (known as histone tail) leading to a more stringent (de-acetylation) or less tight (acetylation) folding of the DNA around the core histones. A second group of enzymatic complexes with ATP-dependent helicase activity allows sliding of the nucleosomes along the genomic DNA, leaving specific DNA regions accessible to transcription or replication factors. Because of the critical function that these enzymatic complexes have in gene regulation and therefore in the control of cell proliferation and differentiation, it is not surprising that in cancer cells their components are frequently targeted by dominant or recessive mutations resulting in altered chromatin structure.

CHROMOSOMAL ABNORMALITIES AND HUMAN CANCER

Cancer is a genetic disease characterized by dominant and recessive gene mutations that

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result in the alteration of normal differentiation and proliferation pathways. Some mutations are inherited and are therefore present in all cells. Many mutations, however, are acquired by one single somatic cell during the lifetime of the organism and are transmitted in clonal pattern to the progeny of the cell. Examples of genetic mutations in cancer are point mutations resulting in inactivation of tumor suppressor proteins (p53 and pRB) or constitutive activation of signal transducers (RAS), chromosomal deletions or duplications, and random or recurring chromosomal rearrangements. Among all these abnormalities, recurring chromosomal translocations are perhaps the most frequent genetic lesion observed by classic cytogenetics and fluorescence in situ hybridization (FISH) in human leukemia and in a subgroup of sarcomas. Each chromosomal translocation is almost invariably associated with only one type of leukemia or sarcoma. This finding, first reported almost 30 years ago for the 9;22 translocation associated with CML, suggested that the breakpoints of the chromosomal translocation are the site of genes critical for the normal differentiation and life-cycle of the affected cell. By using molecular tools, a very large number of genes involved at the breakpoint of chromosomal translocations have been cloned, and it was shown that the rearrangements resulted in alteration of either the level of expression or the biochemical properties of the encoded proteins. Several biochemical and biological studies have confirmed that the alteration of affected proteins play a central role in neoplastic transformation. Chromosomal rearrangements often target proteins that have strong tyrosine kinase activity (either transmembrane receptors or receptor-associated proteins) and nuclear factors that interact with chromatin remodeling enzymes, and result in the expression of a chimeric (or fusion) protein that contains functional domains from both the parental proteins. The motif that is often retained is an oligo- or multimerization domain that mediates protein self-interaction. In the case of chimeric tyrosine kinases, the dimerization leads to continuous self-activation of the kinase in the absence of the proper ligand. However, in totality the major group of recurring genetic abnormalities in human cancers includes point mutations and rearrangement of genes that encode nuclear factors interacting directly or indirectly with enzymatic complexes involved either in histone modification or in chromatin remodeling. The two types of complexes perform distinct functions, which are either the covalent modification of core histones, or the ATP-dependent reconfiguration of chromosomal architecture. Here we will briefly review the normal cellular role of these complexes and describe current models that show how their alteration leads to cellular transformation.

TRANSCRIPTION CO-REGULATORS: DECIDING CELL FATE BY HISTONE ACETYLATION

The activation and repression of gene transcription in response to external stimuli determine the cell fate by initiating programs that are often irreversible and that lead to cell differentiation, proliferation, or cell death. At the heart of transcription regulation is the assembly of multimeric complexes between sequence specific DNA-binding transcription factors and families of transcription co-activators or corepressors. The current models of transcription regulation are based on the ability of these large enzymatic complexes to acetylate specific residues in the histone tails and disrupt the chromatin condensation, hence providing or precluding access to transcription machinery (Fig. 1). Our understanding of how these regulatory complexes reversibly modify the chromatin structure and control gene expression follows the remarkable progress in defining the nuclear hormone receptors as co-regulators of gene transcription. The retinoic acid (RA) receptors RXR and RAR form the functionally active RXR-RAR heterodimer that belongs to the superfamily of nuclear receptors together with glucocorticoid and thyroid receptors. Among them, RAR is the target of virtually all chromosomal translocations associated with acute promyelocytic leukemia (APL) [Melnick and Licht, 1999]. The RA receptors have a modular structure that includes a conserved domain of DNA-binding zinc fingers and a ligand-binding domain that is also necessary for transactivation and hetero-dimerization of the receptors with each other or with other members of the nuclear receptor family (for RXRs only). RXR-RAR is thought to be consistently in the nucleus bound to the cognate DNA sequence (Fig. 2). This is in contrast to other nuclear receptors such as the thyroid hormone

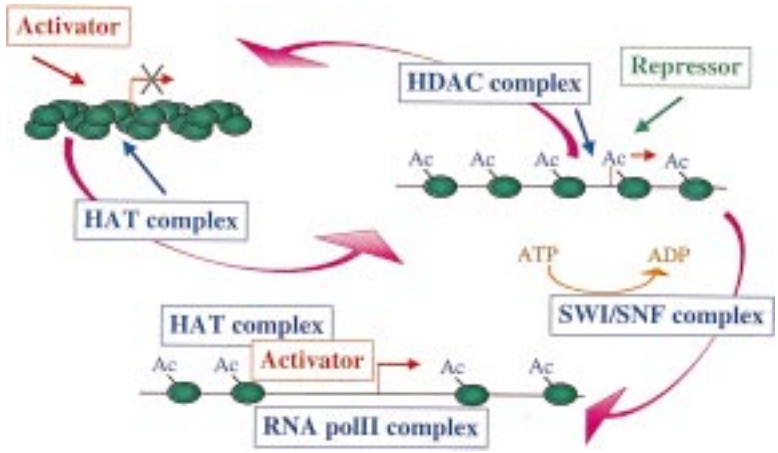

Fig. 1. The reversible transition of the chromatin from a compact state to a relaxed state requires the participation of nucleosome-modifying complexes. The DNA is packed in a highly compacted structure (left) that does not allow promoter transcription (X). After the docking of a transcription activator at the promoter, a histone acetyl-transferase (HAT) complex is recruited, and the acetylation (Ac) of histone tails occurs leading to a relaxed configuration of the chromatin (right). This transition is reversible through the activity of a histone de-acetylation enzymatic complex (HDAC) recruited by a transcription repressor. The activity of a nucleosome remodeling enzymatic complex (SWI/SNF) allows repositioning of the nucleosome and gene transcription.

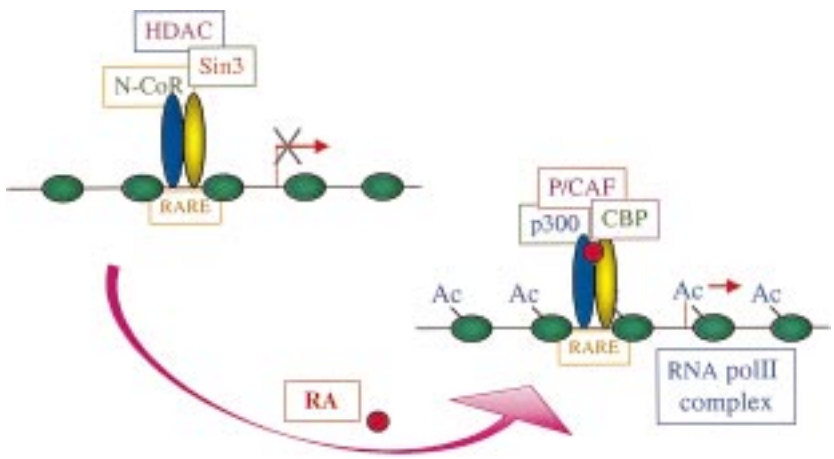

Fig. 2. The retinoic acid receptor regulates gene transcription by recruiting co-activators and corepressors. The heterodimeric RXR-RAR receptor specifically binds to the retinoic acid regulatory elements (RARE). In the absence of the receptor ligand (retinoic acid, RA red dot), the ID1 or ID2 regions of N-Cor or SMRT interact with RXR-RAR. The corepressor complex (N-CoR, Sin 3, and HDAC) is assembled, and the nuclear receptor inhibits gene transcription. When the receptor interacts with the ligand, the affinity of RXR-RAR for the corepressor complex is lower than for the activator complex (p300, P/CAF, and CBP) leading to histone acetylation and gene transcription.

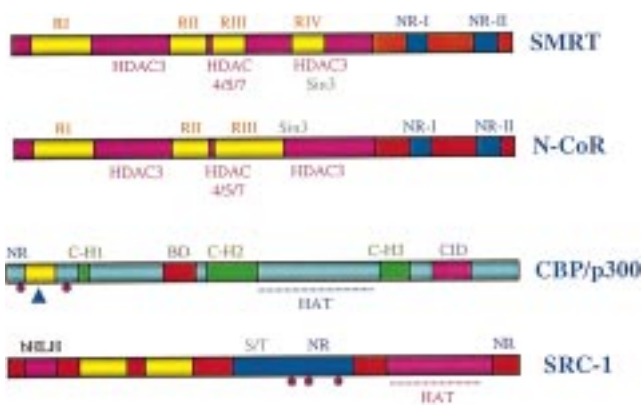

Fig. 3. Structural and functional domains of coregulators. Top: corepressors SMRT and N-CoR. The structural and functional domains of the two proteins are shown as differently colored boxes. Domains function is indicated as follows: repression domains (R); interaction domains with nuclear receptors (NRI and NRII, also known as ID1 and ID2), Sin3, or with HDACs (HDAC1-7). Bottom, the common structural motifs of the co-activators CBP/p300 and SRC-1 shown in the diagram are the helix-loop-helix (HLH) and the LXXLL repeats (red circles). Regions rich in cystine and histidine (CH) and serine/threonine are also indicated. BD: bromo domain. The HAT enzymatic domain is underlined. The blue arrowhead indicates the breakpoint in chromosomal translocations associated with human leukemia.

receptor that, in the absence of ligand, is found in the cytoplasm. Histone de-acetylases (HDACs) are the major players of transcription repression. They hydrolyze the acetyl groups from the lysine residues of the histone proteins thereby leading to a tighter folding of the DNA around the core histones and inhibiting access of transcription machinery to the DNA. Early in vitro studies clarified the role of HDACs in chromatin structure by showing that their inhibition led to the accumulation of acetylated histones [Boffa et al., 1978]. Multiple forms of highly conserved HDACs (HDAC1 to HDAC7) selectively participate to the assembly of large complexes at the promoter site. Members of the corepressor complex either contact directly the transcription factors or are recruited to the promoter by other corepressors that directly interact with the DNA-bound transcription factor. Unliganded RXR-RAR interacts with the DNA retinoic acid response elements (RARE) in promoter sites and is constitutively bound to the highly conserved and closely related corepressors N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoic acid and thyroid hormone receptors). These corepressors are characterized by conserved bipartite nuclear receptor interaction domains and by multiple independent repression domains (Fig. 3). The recruitment of HDAC is often mediated by the adaptor protein mSin3 that interacts with both the corepressors and the HDAC. The assembly of RXR-RAR into an active multimeric repressor complex requires the participation of several other proteins [Alland et al., 1997, Heinzel et al., 1997, Nagy et al., 1997]. According to proposed models, it is thought that the ID1 or ID2 regions of N-CoR or SMRT interact with either the RXR or the RAR subunit, therefore directly recruiting one single corepressor molecule [Jepsen et al., 2000] (Fig. 2 and 3). By using targeted mutagenesis in mice and analysis of N-CoR-null animals, it appears that N-CoR regulation is required for mediation of active repression by specific nuclear receptors and transcription factors on a subset of target genes regulating erythrocytic, thymic and neural systems. However, the assembly of N-CoR and SMRT does not lead invariably to gene repression, and recent findings provide evidence that in a particular context, N-CoR has a role in gene activation of specific retinoic acid elements [Jepsen et al., 2000].

There are remarkable flexibility and swiftness in the manner in which the nuclear receptors recognize intracellular and extracellular stimuli and respond to new biological needs by quickly performing opposite functions such as repression or activation of gene transcription. Structural analysis has clarified the mechanism of response by showing that after binding to the RA ligand, a conformational modification of the ligand-binding domain occurs that disrupts and disassembles the existing corepressor complex and favors the recruitment of co-activators that activate gene transcription. This transition from gene repression to gene activation often results in cell growth and differentiation. This antagonistic role of RXR-RAR as activator or repressor of gene transcription depends on the opposite ways in which the two types of coregulator complexes can modify chromatin condensation by controlling the extent of histone acetylation through their histone acetyltransferase activity (HAT, co-activators) or histone de-acetylase activity (HDAC, corepressors). There are several families of HAT co-activators consisting of highly homologous enzymes that interact with the ligand-binding domain of nuclear receptors in the presence of their ligand. The best-described family includes several proteins with high homology to p160. These enzymes display structural features similar to those identified in the steroid receptor co-activator 1 (SRC-1) (Fig. 3). The members are highly homologous but can be divided further into three groups, SCR/N-CoA, TIF2/GRIP/N-CoA2, and pCIP/ACTR/TRAM-1, based on higher homology stringency [Chen et al., 1995; Onate et al., 1995; Voegel et al., 1996; Torchia et al., 1997; Voegel et al., 1998; Collingwood et al., 1999]. They are distinguished by multiple short helical motif LXXLL (red dots in Fig. 3) sufficient for the interaction with the amphipathic helix motif AF1 of the RA receptor ligand-binding domain (Figs. 3 and 4) [Heery et al., 1997; Torchia et al., 1997]. The LXXLL motif is also necessary for the interaction with members of other families of co-activators [Heery et al., 1997; Torchia et al., 1998] leading to the assembly of a multimeric co-activator complex with the receptor. Mutations of the LXXLL motif abrogates the assembly of the complex and the transactivation property of the nuclear receptors [Collingwood et al., 1997, 1998; Feng et al., 1998; Nolte et al., 1998].

P300 and CBP form a second family of HAT enzymes with the ability to change chromatin architecture by acetylation of histones and the histone-related protein HMGI (Y). They can also acetylate non-chromatin proteins such as the transcription factors p53, GATA1, EKLF and the basal transcription factors TFIIβ and TFIIF [Imhof et al., 1997; Boyes et al., 1998; Munshi et al., 1998; Sakaguchi et al., 1998; Zhang and Bieker, 1998]. Both p300 and CBP bind to nuclear receptors in a ligand-dependent manner. They decondense chromatin and facilitate the binding of the transcription complex to the core promoter thereby activating transcription [Chakravarti et al., 1996]. Recent studies suggest that CBP and p300 are components of a large protein complex containing additional HAT enzymes. They synergize with SRC-1 [Smith et al., 1996], TIF2 [Voegel et al., 1996, 1998], or with both SRC-1 and P/CAF [Yang et al., 1996; Jenster et al., 1997; Blanco et al., 1998; Korzus et al., 1998] to enhance the activity of transcription activators. CBP, P300 and their associated factor P/CAF have multiple interaction domains that are required for specific binding to a remarkable variety of proteins, from transcription factors, to transforming proteins, to tumor suppressors. Critical CBP-dependent activator sites can be located over 1 kb from the transcription start site, and it is difficult to imagine how CBP-containing complexes can decondense the core promoter at such distance [Mannervik et al., 1999]. P300 was first characterized as a target of the viral transforming protein E1A, suggesting that it could have an essential role in the control of cell cycle and cell proliferation [Stein et al., 1990]. This hypothesis is supported by the finding that p300, CBP, and the CBP/p300-binding protein TIF2 are rearranged by recurring chromosomal translocations that presumably alter their normal functions [Borrow et al., 1996; Ida et al., 1997; Sobulo et al., 1997; Satake et al., 1997; Carapeti et al., 1998; Liang et al., 1998] and by recent evidence showing that the HAT activity of CBP increases at the G1/S boundary after phosphorylation by cyclin-dependent kinases [Ait-Si-Ali et al., 1998;]. Several reports indicate that p300 and P/CAF could play a central role in the control of G1/S by regulating the stability and the localization of p53 after it is acetylated [Gu and Roeder, 1997; Lill et al., 1997; Sakaguchi et al., 1998; Ait-Si-Ali et al., 2000; Magnaghi-Jaulin et al., 2000].

CHIMERIC TRANSCRIPTION FACTORS: CHROMATIN CONDENSATION AND CELL TRANSFORMATION

In APL, the chromosomal rearrangement of RARα, located on chromosome band 17q21, leads to gene fusion and to the expression of a chimeric receptor. The t(15;17), resulting in the chimeric receptor PML-RARα, was one of the first translocations associated with leukemia to be identified by cytogenetic analysis [Rowley et al., 1977] and the first one involving RARα to be cloned [de The et al., 1990]. Five chromosomal translocations involving RARα have been described so far, and five different genes, PML [98% of cases, Grignani et al., 1994], PLZF, NPM, NuMA, and STAT5b are rearranged upstream of RARα and are expressed as chimeric RARα receptors [for recent reviews see Lin et al., 1999; Melnick and Licht, 1999]. Aside from PLZF-RARα for which only one form was detected, there are multiple breakpoints or splicing isoforms resulting in alternative chimeric proteins. In any case, however, the result of the chromosomal translocation is the expression of a chimeric RA receptor that contains the same region of RARα consisting of the DNA- and the ligand-binding domains fused to a unique N-terminus sequence derived from the partner protein (Fig. 4). The partner genes encode proteins with different functions: PML and PLZF are growth suppressors, NPM is a ribonucleoprotein implicated in DNA recombination, NuMA seems to have a role in mitosis, and STAT5b is a signal transducer [for recent reviews see Lin et al., 1999; Melnick and Licht, 1999]. The only obvious common feature shared by the five partner proteins rearranged with RARα is the presence of a dimerization domain at the N-terminus. This domain has the ability to promote homodimerization of the chimeric receptor through a coiled-coil motif (PML) or through other less well defined protein-protein interaction domains [Dong et al., 1996; Cheng et al., 1999]. Independent of the partner gene, the phenotypes of leukemia with RARα rearrangement are undistinguishable, strongly suggesting that the disruption of the RARα signaling is the crucial step for the pathogenesis of the disease. Surprisingly, however, the response of APL patients to all-trans retinoic acid (ATRA), which is the pharmacological agent of choice in APL, is strikingly strong in all cases except for patients who have a t(11;17),

encoding PLZF-RARα. Therefore, the prognosis and the success of the ATRA treatment depends on the type of translocation identified in the APL cells. The clinical response in the APL patients is accompanied by successful myeloid differentiation of APL cells. This treatment requires very high doses of ATRA and is achieved with a pharmacological (10^{-6} mol/L) rather than physiological (10^{-9} mol/L) concentration of ATRA. The biochemical basis of the remarkable difference between PLZF-RARα, for which no response to ATRA is observed, and the other cases is slowly being unraveled. In normal cells, the wild type RXR and RARα form a heterodimer associated with the corepressor complex and bound to RARE; in the absence of the ligand, RXR/RARα represses the transcription of genes necessary for cell differentiation. In presence of the ligand, the repressor complex is released and the nuclear receptor recruits the co-activator complex, resulting in activation of genes necessary for cell differentiation. The chimeric receptor N/RARα, where N is any one of the partner genes rearranged with the RARα receptor in APL, is also bound to RARE. However, in absence of the RA ligand, the chimeric receptor N/RARα has a much higher affinity for SMRT and N-CoR than the wild type RXR-RARα, and is therefore a much stronger repressor that requires higher concentration of ligand to release the corepressor complex [Grignani et al., 1993; He et al., 1999]. For APL patients with PML/RARα, there are additional events that contribute to their positive response to ATRA treatment. ATRA leads to degradation of PML-RARα itself [Raelson et al., 1996; Yoshida et al., 1996] and upregulation of RARα expression, thereby facilitating the normal differentiation pathways of the APL cells [Chomienne et al., 1991]. In contrast, patients with a t(11;17) and expression of PLZF-RARα fail ATRA treatment. The basis of this unusual response puzzled scientists for several years, until it was shown that the N-terminus region of PLZF fused to RARα also interacts very strongly with the components of the corepressor complex. The interaction is RA-independent and therefore does not result in dissociation of the corepressor complex even at high level of ATRA, explaining the lack of clinical response to ATRA therapy in the patients [Hong et al., 1997; Grignani et al., 1998; Guidez et al., 1998; Lin et al., 1998]. These data did not satisfactorily explain why PML-RARα is a better repressor than RARα and requires higher concentration of ligand to release the corepressor complex (10^{-6} mol/L vs. 10^{-9} mol/L). The biochemical basis of the enhanced repression of PML-RARα was elucidated in recent reports showing that the coiled-coil domain of PML leads to a homodimer (PML-RARα/PML-RARα), rather than to a heterodimer (RXR-RARα/PML-RARα) with stronger affinity for the N-CoR/SMRT complex. Indeed, stoichiometric analysis of the PML/RARα-SMRT interaction found that each PML/RARα subunit can recruit SMRT, in contrast to the normal receptor RXR-RARα in which both subunits interact together with only one SMRT molecule, thus leading to stronger repressor property [Lin and Evans, 2000]. Two lines of evidence confirm these findings: a dimerization-deficient PML/RARα mutant regains normal corepressor binding profile and fails to block RA signaling [Lin and Evans, 2000]; secondly, several forced-homodimerization mutants of RARα alone are sufficient to recapitulate many functions of PML/RARα [Lin and Evans, 2000]. These combined findings provided an explanation for the clinical resistance to ATRA-differentiation therapy observed in patients with PLZF/RARα [Licht et al., 1995; Lin and Evans, 2000]. In addition, they suggest that the powerful transforming property of aberrantly expressed PML/RARα and PLZF/RARα relates to their ability to recruit the transcription corepressor complex and to maintain the chromatin in the de-acetylated state, therefore resulting in a condensed architecture not open to transcription. On a more important clinical level, it was proposed that a combined therapy with ATRA and an HDAC inhibitor (trichostatin-A, butyrate) could be successful for t(11;17) patients and could restore the normal RAR signaling pathway [Warrell et al., 1998].

The overwhelming correlation between disruption of RAR signaling due to chromosomal translocations and leukemia links very strongly the histone deacetylase-mediated transcription repression with the pathogenesis of leukemias. Indeed, other chromosomal translocations rearrange genes encoding transcription factors that recruit corepressors. The nuclear factor AML1 (RUNX1) located on chromosome band 21q22 is fused to the zinc finger nuclear factor ETO in the t(8;21) associated with acute myeloid leukemia (AML) subtype M2 [Miyoshi et al., 1991] and to the ETS-protein TEL (ETV6) in the t(12;21)

associated with childhood leukemia [Golub et al., 1995; Romana et al., 1995]. AML1 is a member of a DNA-binding family of evolutionarily conserved proteins and is essential for murine fetal hematopoiesis. AML1 does not multimerize but heterodimerizes with the CBFβ protein to form the transcriptionally active factor CBF. The DNA-interacting domain at the N-terminus of AML1, known as RUNT domain, specifically binds to its cognate DNA site and regulates gene transcription in synergy with other nuclear factors (ETS proteins or C/EBP factors) that occupy adjacent sites in the promoter. The C-terminus of AML1 contains domains that interact with corepressors and co-activators, and a nuclear matrix targeting signal (NMTS) necessary for localization to a subset of nuclear foci that contain hyper-phosphorylated RNA polymerase II [McNeil et al., 1999]. A diagram of AML1, ETO, TEL, and the chimeric proteins are shown in Figure 5. The 8;21 chromosomal translocation results in a chimeric gene that encodes the N-terminus of AML1 including the DNA-binding domain fused in frame to almost the entire ETO, a zinc-finger protein that physically interacts with N-CoR and SMRT [Gelmetti et al., 1998; Lutterbach et al., 1998; Wang et al., 1998]. In addition, ETO contains two homodimerization domains, the distal coiled-coil motif P1, and the amphipathic alpha helix P2 [Minucci et al., 2000]. The chimeric protein, AML1/ETO, has lost the C-terminus of AML1 and the ability to interact with p300/CBP, but has acquired the potential to form homodimers and to recruit multiple copies of N-CoR and SMRT through the ETO domains [Minucci et al., 2000]. Indeed, AML1/ETO forms a high molecular size complex that disappears when the P1 and P2 domains are deleted [Minucci et al., 2000]. Assembly of the complex correlates with an increase in recruitment of N-CoR [Minucci et al., 2000]. The high molecular size complex is a very strong repressor necessary to block the hematopoietic differentiation, therefore reinforcing the correlation between an inappropriate multimeric repressor complex and a differentiation block leading to leukemic transformation. Deletions of either the N-CoR interacting site or the dimerization motifs impairs the capacity of the chimeric protein to block differentiation of primary hematopoietic precursors, confirming that, as for PML-RARα a biological correlation exists between disregulated repression and loss of differentiation. The fusion protein TEL/AML1 contains the entire AML1 fused downstream of a truncated form of TEL that lack the ETS-DNA-binding domain (Fig. 5). TEL is a transcription factor that interacts with UBC9 and is modified by covalent addition of SUMO1 [Chakrabarti et al., 1999A]. SUMO1-modified TEL localizes to nuclear structures [Chakrabarti et al., 2000]. In addition, TEL contains two independent repressor domains: the HLH or pointed (PNT) domain at the N-terminus and the central region between the HLH domain and the ETS domain [Chakrabarti et al., 1999A]. The HLH region is also a protein interaction region that oligomerizes with itself and with FLI1. The central region of TEL interacts with several corepressors [Chakrabarti and Nucifora, 1999B; Fenrick et al., 1999]. Thus, like ETO, TEL is characterized by an oligomerization region and by a repression domain capable of recruiting corepressor. There are no conclusive data yet demonstrating that the leukemic property of TEL/AML1 depends on the ability of the fusion protein to multimerize. However, the results with the chimeric RARα receptors and with AML1/ETO suggest that the efficient recruitment of N-CoR/HDAC complex and the inappropriate formation of multimeric complexes are required to activate the oncogenic potential of the chimeric proteins. The non-physiological recruitment of corepressors could lead to a chromatin configuration of target promoters refractory to activate signals from other cis-acting elements. The results pinpoint to a potential novel class of drugs for leukemia treatments that target and inhibit HDAC. The feasibility of using these drugs to restore the differentiation of the leukemic cells has been tested in cell lines, in which preliminary results strongly indicate that the AML1-ETO leukemic cells can be successfully differentiated by treatment with HDAC-inhibiting agents such as trichostatin-A [Wang et al., 1999].

CHIMERIC CO-ACTIVATORS: CHROMATIN RELAXATION AND CANCER

Transcription co-activators are at the heart of biological pathways and serve as interpreters of physiological stimuli to regulate cell cycle, DNA repair, differentiation, and apoptosis. Thus, it is not surprising that their functions are also altered in cancer. Three co-activators, TIF2

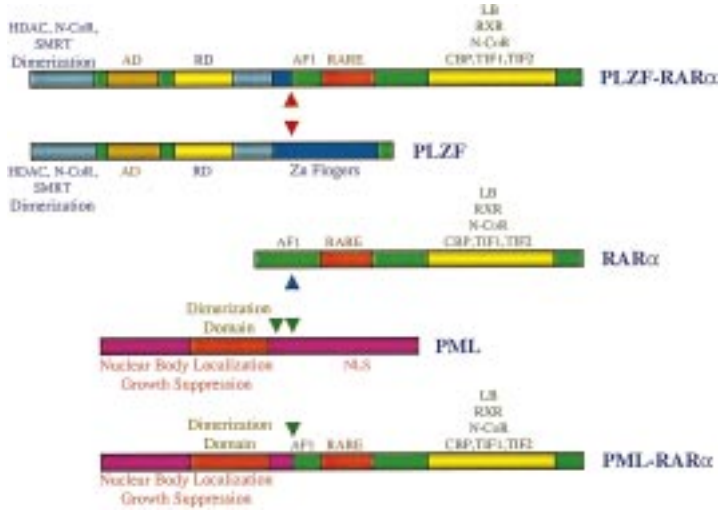

Fig. 4. Diagram of the retinoic acid receptor RAR (center), the PML and PLZF proteins with which is rearranged following chromosomal translocation, and the chimeric products (top and bottom). RARE is the DNA-binding domain. The regions interacting with coregulators, with the ligand (LB), and with RXR are also indicated. PLZF has a RA-independent repression domain and a dimerization domain at the N-terminus maintained in the chimeric protein (bottom). The chromosomal breakpoints are indicated by a red arrow (PLZF), a blue arrow (RAR), and by two green arrows for PML that has two breakpoint regions distal to the dimerization region and the nuclear body localization. NLS: nuclear localization signal.

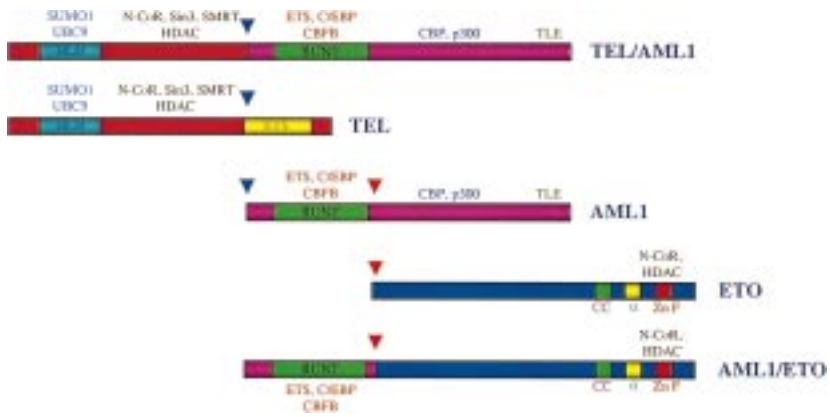

Fig. 5.

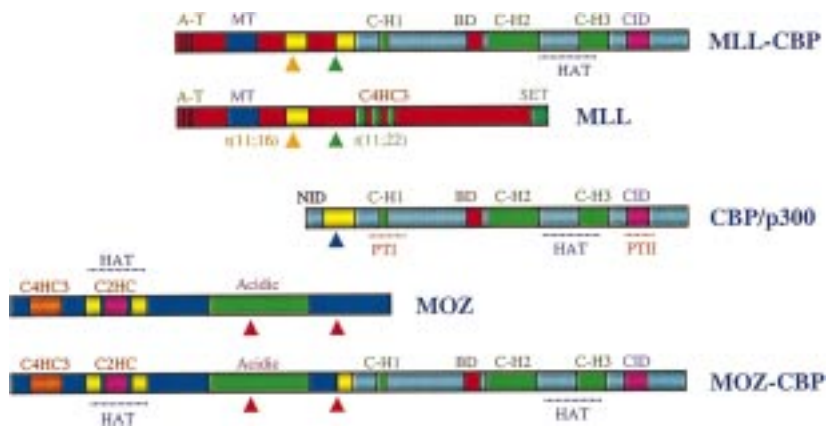

Fig. 6.

(encoding a nuclear receptor co-activator that binds to CBP and p300), CBP, and p300 are directly rearranged by chromosomal translocations in leukemia. This mechanism of leukemogenesis is in contrast to that described for corepressors, which are recruited unaltered to core promoters by chimeric transcription factors. Mutational analysis has shown that the conserved HAT domain of CBP possesses intrinsic HAT activity and supports gene transcription, demonstrating a clear correlation between the two properties. Targeted mutation and inactivation studies have confirmed that CBP and p300 are necessary for embryonic development. CBP+/− or p300+/− mice display aberration of embryonic development and somatic growth, suggesting that there is a minimum critical dosage of each protein necessary for normal development [Tanaka et al., 1997; Yao et al., 1998]. The requirement of a threshold level of expression is observed also in humans. P300 missense mutations have been reported in colorectal and gastric carcinomas [Muraoka et al., 1996], and if one functional allele of CBP is inactivated, an autosomal dominant condition (Rubinstein-Taybi syndrome) appears that is characterized by cranial and digital malformation, mental retardation, and predisposition to cancer [Giles et al., 1998]. CBP interacts with many proteins involved in the control of cell cycle and cell proliferation (see Fig. 6), supporting the hypothesis that co-activators could have a dominant role in regulating cell proliferation. So far, co-activators are rearranged in four cloned chromosomal translocations associated with leukemia. In the t(8;16) and inv(8), respectively CBP or TIF2 are rearranged downstream of the MOZ gene (monocytic leukemia zinc finger) [Borrow et al., 1996; Carapeti et al., 1998; Liang et al., 1998]. MOZ (Fig. 6) has multiple domains including a C4HC3 PHD finger domain, a C2HC atypical zinc finger domain, and a region with limited homology to the active acetyl-CoA binding domain of several HAT proteins from yeast (Sas) to Drosophila (Mof) [Reifsnyder et al., 1996; Ehrenhofer-Murray et al., 1997; Neuwald and Landsman, 1997; Dutnall et al., 1998; Wolf et al., 1998]. However there are no definitive data showing that MOZ has HAT activity. In those inv(8) breakpoint junctions that have been cloned, the chromosomal breakpoints in the MOZ gene are very close. The fusion products encode a protein consisting of the N-terminus of MOZ fused to the C-terminus of TIF2 that contains the CBP-interacting domain and an activation domain. In the t(8;16), the N-terminus of MOZ is fused to almost the entire open reading frame of CBP. The resulting fusion protein is very large and includes the three cystine/histidine-rich regions C/H1-3, the C-terminus activation domain, the bromo domain, and the HAT domain of CBP. Diagrams of chimeric proteins that involve MOZ, MLL, CBP, and p300 are shown in Figure 6. CBP is also rearranged in the t(11;16) which is associated with therapy-related leukemia [Satake et al., 1997; Sobulo et al., 1997]. Two breakpoints have been described for CBP and in both cases the resulting proteins contain the region from the bromo domain to the C-terminus. The gene that is fused upstream of CBP is MLL (also

Fig. 5. AML1 and chromosomal rearrangement partners. AML1 (center) contains two major functional domains, the DNA-binding Runt domain at the N-terminus and the activation (CBP, p300) and repression (TLE) domains. The Runt domain interacts with ETS and C/EBP proteins and with CBFB. AML1 is rearranged in the t(12;21) with TEL (blue arrowheads indicate breakpoints). TEL is a transcription repressor that interacts with corepressors and is modified by SUMO1. The HLH domain of TEL is a multimerization domain. The chimeric protein TEL/AML1 (top) contains the entire AML1 open reading frame. In the t(8;21), AML1 is split between the Runt and the transactivation domains (red arrowheads) and is fused to almost the entire ETO. Functional and interaction domains of ETO are indicated. In the chimeric protein AML1/ETO (bottom), the C-terminus region of AML1 interacting with CBP/p300 is replaced by the multimerization domain (coiled coil, CC) and the C-terminus of ETO that interacts with corepressors. Additional domains of ETO are ZnF: zinc fingers, α: alpha helical structure.

Fig. 6. CBP and chromosomal rearrangement partners. Structural and enzymatic domains of CBP (center) are indicated as follows: NID: nuclear receptor interacting domain, CID: CDK interacting domain, C-H1, -2, -3: cysteine and histidine-rich regions, BD: bromo domain, HAT: histone acetyltransferase domain, PTI: protein-interacting region that binds to CREB, c-Jun, c-Myc, Sap-1, Elk-1, SREBP, Tax; PTII: protein-interacting region that binds to E1A, Stat1a, TFIIB, pCAF, RNA helicase A, Myo-D, C-Fos, SV40 large T antigen, p53, E2F, and Tag., the blue arrowhead indicates the breakpoint. Only the most relevant domains of MLL are shown: A-T: A/T hooks; MT, methyltransferase homology domain; C4HC3, PHD fingers; SET, homology region to the SET domain that interacts with the SNI/SNF complex. The green arrowhead indicates the breakpoint in the t(11;22); the orange arrowhead indicates the breakpoint in the t(11;16). In the lower part of the figure, domains of MOZ and the chromosomal breakpoints (red arrowheads) are shown.

known as HRX and ALL), and is involved also in the t(11;22) with p300 [Ida et al., 1997]. From a functional point of view, the breakpoints of CBP and p300 are analogous and include the same functional domains, indicating that the region from the bromo domain to the C-terminus is necessary for the pathogenesis of the leukemia. Indeed, analysis of murine bone marrow expressing various C-terminus and internal CBP deletion mutants of an MLL/CBP chimeric protein confirms that the bromo domain and the HAT domain are necessary and sufficient to transform the murine bone marrow and to induce leukemia in recipient mice [Lavau et al., 2000]. MLL encodes a very large protein conserved through evolution from man to Drosophila. Inactivation of the MLL homolog Trx in flies results in patterning defects and homeotic transformation suggesting that Trx plays an essential role in regulation of developmentally expressed genes. In the mouse, the abrogation of Mll results in embryonic death due to a variety of developmental defects. Mll−/+ mice develop hematopoietic stem cell abnormalities and bidirectional homeotic-like transformation similar to those observed in Trx-mutant Drosophila, confirming that the mammalian homolog has a development regulatory role and suggesting that a limiting critical expression of the protein must be maintained for normal development. This hypothesis is confirmed by the reported aberrant expression of the Hox genes in Mll-null murine embryonic fibroblasts. MLL is a complex protein with several functional domains. In addition to transcription regulation domains, at the C-terminus MLL contains a SET domain that interacts with SNF5/BAF47, a component of the SWI/SNF complex involved in chromatin remodeling and transcription regulation [Pollard and Peterson, 1998; Rozenblatt-Rosen et al., 1998]. The MLL chromosomal breakpoints in the t(11;16) and t(11;22) are not identical, however they retain the MLL regions with homology to methyltransferase and three A/T hook repeats, originally described in HMGI(Y) proteins and histones. The A/T hook motif is presumed to bind non specifically to nucleosomal DNA rather than to a specific DNA sequence. The function of MLL is unknown. Based on its functional motifs, it is suggested that MLL could be involved in chromatin remodeling. Thus, it is possible that the chimeric proteins resulting from 11q23 translocations could perturb the chromatin structure of regions that are targeted by MLL and inappropriately regulate the expression of developmental genes.

ROLE OF SWI/SNF IN CANCER

It has been known for several years that the nucleosomes play a very important and active role in the transcription regulation of eukaryotic genes. There are several ATP-dependent protein complexes that regulate nucleosome remodeling and transcription regulation by disrupting or altering the association of core histones with DNA. Analysis of genetic mutations in yeast first showed that non-DNA binding proteins are required for the activity of several promoters involved in biochemical pathways. Complementation analysis with extragenic genes led to the identification of a group of proteins necessary to repair defects in mating type switching (SWI) and sucrose fermentation (SNF: sucrose non fermentor) [Armstrong and Emerson, 1998]. These proteins are highly conserved from yeast to Drosophila to man and are components of a 2 MDa multimeric complex known as SWI/SNF that displays ATP-dependent ability to modify the nucleosomal organization [Laurent et al., 1991; Hirschhorn et al., 1992; Peterson and Herskowitz, 1992; Cairns et al., 1994; Treich et al., 1995; Cairns et al., 1996; Wilson et al., 1996; Bazett-Jones et al., 1999]. Further studies in yeast have pointed out to other evolutionarily conserved complexes that have ATPse activity, such as the yeast RCS, the Drosophila NURF, CHRAC, and ACF, and the mammalian NRD/NuRD [Tsukiyama et al., 1995; Cairns et al., 1996b; Ito et al., 1997; Varga-Weisz et al., 1997; Tong et al., 1998; Wade et al., 1998; Zhang et al., 1998]. These complexes have unique subunits, but they all include a component with a very conserved domain that has DNA-dependent ATPase activity. Two models have been proposed to explain how these complexes modify the nucleosomal structure. The first model suggests that the ATP energy is used to isomerize the nucleosome structure into a different structure with the same components but with altered histone-DNA contacts. The central proposition of this model is that an altered nucleosome structure could be stable in the absence of the remodeling complex. The second model proposes that ATP hydrolysis could be used to promote continuous movement

of the complex around the nucleosomal DNA, and the complex could force the DNA away from the histone core as it moves [Workman and Kingston, 1998]. There are no clear data supporting one of the two models unequivocally. The latter model is however weakened by the finding that ATP is not required for maintaining the disrupted state of the nucleosome [Imbalzano et al., 1996].

Components of the SWI/SNF complex have been linked to cancer. One of them is the subunit with ATPase activity. This enzyme has homology to known DNA helicases, and in yeast is known as SWI2/SNF2, in Drosophila as Brm, and in man there are two homologous proteins known as BRG1 and BRM. It was shown that BRG1 and BRM remodel the nucleosome structure without the participation of other subunits [Phelan et al., 1999; Vignali et al., 2000]. Furthermore, in vitro studies showed that the addition of three other SWI/SNF components, SNF5/INI1, BAF155, and BAF170, increases the efficiency of nucleosome disruption to that of the intact SWI/SNF complex [Phelan et al., 1999]. However, there has been a failure to demonstrate conclusively that BRM and BRG1 have helicase activity, and single stranded DNA has not been detected within disrupted nucleosomes [Cote et al., 1994, 1998]. Both BRG1 and BRM interact with the tumor suppressor protein pRB, whereas BRG1 interacts only with the tumor suppressor protein BRCA1 [Trouche et al., 1997; Bochar et al., 2000]. The importance of BRG1 in maintaining the normal function of pRB was demonstrated in studies showing that overexpression of BRG1 in cell lines co-operates with pRB and causes growth inhibition that is dependent on the interaction with pRB [Dunaief et al., 1994; Imbalzano et al., 1994; Khavari et al., 1993; Kwon et al., 1994; Trouche et al., 1997]. Furthermore, BRG1 enhances pRB-mediated inhibition of E2F transcription [Trouche et al., 1997], and is required for pRB-mediated signaling to critical downstream effectors and subsequent cell cycle arrest [Strobeck et al., 2000]. Loss of BRG1 function in tumor cells renders them resistant to the antiproliferative activity of pRB, thus revealing a new mechanism by which tumor cells could attain growth advantage [Strobeck et al., 2000]. Previously, a large body of evidence had pointed out the role of BRCA1 in transcription control, and it was shown that BRCA1 acts as a co-activator in a p53-mediated transcription probably through interaction with CBP/p300 [Pao et al., 2000]. Now BRCA1 has been identified as a component of the SWI/SNF complex itself, providing a mechanism by which BRCA1 regulates transcription through modulation of chromatin structure [Bochar et al., 2000].

The evidence of BRG1 and BRM involvement in cancer is indirect and it is limited to interaction with known tumor suppressors like pRB and BRCA1, which are often mutated or deleted in cancer. However, molecular analyses of rhabdoid tumors has provided clear evidence that the alteration of SWI/SNF subunits can be a direct cause of neoplastic transformation. Rhabdoid tumor is one of the most aggressive types of childhood cancers. It was described initially as a variant of Wilms tumor on the basis of its location in the kidney and the phenotypic appearance of the cells. Later the tumors were observed in various organs, including the CNS, lung, and liver, and they were recognized as a separate malignancy. In the majority of cases, the molecular and karyotypic analyses of the tumor cells indicate that deletion and loss of heterozygosity on chromosome 22 band 11 occur [Versteege et al., 1998]. Using a panel of rhabdoid tumor cell lines, a careful map of the minimum, common deleted region was obtained, and a gene, SNF5/INI1 an inhibitor of the HIV integrase, was identified by positional cloning strategy [Versteege et al., 1998]. SNF5/INI1 is a homolog of the yeast Swi5. SNF5/INI1 interacts with BRM and is a subunit of the SWI/SNF complex. In rhabdoid tumor patients and cell lines, several mutations targeting SNF5/INI1, such as gene deletion or frame shift or nonsense point mutation, have been reported. These mutations affect the conserved C-terminus of the protein and result in a truncated protein that has lost the domain necessary for interaction with BRM. The consistent association of SNF5/INI1 mutations with the development of rhabdoid tumors clearly indicates that the SWI/SNF complex is important in the pathogenesis of the disease, but how SNF5/INI1 alteration leads to cancer is unknown. Because BRM and SNF5/INI1 interact, it is possible that the SWI/SNF complex is involved in control of cell replication. Thus, truncations or deletions of SNF5/INI1 could affect the function of pRB in the regulation of E2F and lead to inhibition of G1 arrest. It has been reported that SWI/SNF affects the function of

nuclear receptors by increasing their activating ability [Ichinose et al., 1997; Trouche et al., 1997; Fryer and Archer, 1998] resulting in cell cycle arrest. Thus, alternatively, the loss of SWI/SNF function could lead to inappropriate chromatin remodeling and loss of expression of genes that inhibit cell cycle progression. As SNF5/INI1 mutations predispose to cancer, it is possible that SNF5/INI1 has tumor suppressor properties, although a conclusive and direct involvement of this protein in cell cycle regulation has not been shown.

CONCLUSION

The identification of proteins involved in chromatin structure as targets of genetic lesions associated with human cancers has clearly indicated that the biochemical alteration of these factors is a dominant event in the pathogenesis of the disease. With the progress of our understanding of chromatin architecture, it appears that the physiological unbalance of coregulator and chromatin remodeling complexes brought by mutated factors leads to the inappropriate alteration of the chromatin structure and to abnormal gene regulation. This novel view of illegitimate chromatin restructuring as a cause of human disease is supported by the findings of many investigators and provides us with new and exciting means to attack and control human disease. The results of cell line and murine studies proposing the use of HDAC inhibitors to control rearranged transcription factors have been very encouraging, and the first clinical trials for leukemia patients with a t(15;17) or t(8;21) are under way. At this time, however, even in preliminary in vitro systems there are no drugs effective yet to reverse the effects of activators and components of the chromatin remodeling complexes that have been altered by genetic lesions. It is clear, however, that a novel road has been mapped, and eventually new "designer" drugs will be synthesized and available to repair many of the genetic lesions that contribute to human cancers.

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