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Novel mammalian chromatin reconstitution *in vivo*

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A recent breakthrough in cancer research is the discovery that various transcription factors (TFs) that are involved in cancer, such as p53 and pRB, BRCA-1, AML-1/ETO, PML-RAR-a, etc., actually regulate cancer by modifying chromatin. However, the exact mechanism of TF-mediated chromatin modification in mammalian cells *in vivo* is not known. For example, it is not known whether a specific TF modifies specific histones and specific amino acids within the histones during this process or whether all of the TFs modify the same histones. Because histone modification is one of the ultimate steps in the regulatory circuits of these TFs, such studies may yield a new approach to cancer therapy. For instance, small molecules can be synthesized that can either augment or block specific TF-histone interactions and thus, regulate cancer. The major road-block in determining such TF-histone interactions arises because mammalian cells *in vivo* are not amenable to introduction of mutant histones. By microinjecting plasmid-encoded genes and transcriptional regulatory sequences in early mouse embryos and examining the genes' expression, we have developed a novel system in which chromatin modulation can now be studied in mammals. Results from our and other laboratories suggested that expression from such microinjected plasmids reflects physiological regulation present in these embryos. Utilizing this system, we previously observed that the paternal pronuclei of one-cell embryos do not contain chromatin structure. In contrast, chromatin structure was observed in two-cell embryos. Our recent experiments further indicated that co-injection of purified histones and a plasmid-encoded reporter gene into the paternal pronuclei of one-cell embryos at a specific histone-DNA concentration could reconstitute the behavior observed in two-cell embryos, both structurally (chromatin assembly) and functionally (acquisition of promoter repression and subsequent relief of this repression by functional enhancers or by histone deacetylase inhibitors). Thus, injection of exogenous histones into one-cell embryos faithfully reproduced the chromatin-mediated transcription observed in two-cell embryos and provided the first *in vivo* mammalian model system in which the role of individual histones, and particular domains within the histones that are specifically targeted during a given TF-mediated enhancer function, can be examined using purified mutant histones.

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Novel peptidomimetic inhibitors of Stat3 signaling and oncogenesis

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One member of the family of Signal Transducer and Activator of Transcription (STAT) proteins, Stat3, participates in malignant transformation. The critical role of Stat3 in the growth and survival of human tumor cells provides a valid basis for targeting Stat3 for development of novel inhibitors. We previously identified a Stat3 SH2 domain-binding peptide, PY*LKTK and its tripeptide derivatives, PY*L or AY*L (where Y* represents phosphotyrosine), which were demonstrated to inhibit Stat3 signaling and oncogenesis. Here we report novel tripeptide mimics that have been developed for improved selectivity and efficacy with regard to inhibition of Stat3 activity. The presence of these peptidomimetic compounds in nuclear extracts results in a dose-dependent decrease in the level of Stat3 DNA-binding activity *in vitro*, with efficacies that are five- to ten-fold higher than previously obtained for tripeptides. In whole cells, a representative peptidomimetic identified as JSK 610 selectively suppresses constitutive Stat3 activation and transcriptional activity in Src-transformed fibroblasts, as well as in human breast and lung carcinoma cells that express constitutively-active Stat3. In a manner that reflects inhibition of aberrant Stat3 activity, JSK 610 inhibits proliferation and induces apoptosis of transformed cells. Altogether, we present evidence for novel peptidomimetic-based specific inhibition of Stat3 signaling, and demonstrate the profound biological outcome of growth inhibition and induction of apoptosis in relevant model human tumor cell lines.

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Insight into the mode of action of rViscumin through transcriptional profiling

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Transcriptional profiling is a suitable tool for the identification of new drug candidates for applications in oncology and immunotherapy. Once a novel drug is identified the technique can also be used to obtain detailed information on the mode-of-action. To this aim, a target-oriented cDNA array system (onco-set) was used for the analysis of drug induced transcriptional changes in various intra- and intercellular signal transduction pathways leading e.g. to apoptosis, cell-cycle regulation or cytokine production. We set up an array-based classification system for the molecular characterisation of the novel anti cancer drug rViscumin and compared it to profiles from known cytostatic or antibiotic drugs in a surrogate cell system. The ribosome inactivating protein rViscumin is a potent apoptosis inducing drug with immunomodulatory and anticancer activity *in vitro* and *in vivo*. rViscumin is a recombinant heterodimeric plant derived protein which is currently in clinical development. We applied this drug to a model cell system (THP-1 human monocytic cell line) and compared the expression profile changes induced by standard cytostatic drugs, i.e. Taxol, Hycamtin and Doxorubicin to that induced by rViscumin. In a time course analysis the transcriptional changes with a 24 h interval after the start of treatment were monitored. In rViscumin treated cells the eucaryotic initiation factor-2 (eIF-2, playing a role in regulation of translation) was downregulated by a factor of 5 whereas apoptosis related gene products as metallothionein, DNAse 1 precursor or Adducin-1 alpha were upregulated by at least a factor of 4 compared to the untreated control. Genes regulated in THP-1 cells treated with e.g. Taxol were different and included a downregulation of MAPK2 / Erk2 and G2 / mitotic cyclin B. rViscumin in this setup showed a unique transcription profile and its mode of action can be distinguished from the mode of action of any of the other anticancer drugs tested. These data are in good correlation with the results from a COMPARE analysis based on data from the NCI 60 cell line screening results. The results gave new insight into the molecular mechanism of rViscumin and lead to the identification of predictor gene classes, which can potentially be utilised for monitoring clinical responses. Array-based transcriptional profiling using the onco-set gene collection is a valuable tool for screening and characterisation of novel anti cancer drugs.

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Preclinical efficacy, toxicology and pharmacokinetics of NVP-LAQ824, a novel synthetic histone deacetylase inhibitor

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Reversible acetylation of nuclear histones is a major regulator of gene expression which may act by altering accessibility of transcription factors to DNA by changing higher order structures in the nucleosome. Cell specific patterns of gene expression result from a balance between the competing activity of histone acetyl transferases (HAT) and histone deacetylases (HDAC). Perturbations of this balance have been linked to cancer. Small molecule inhibitors of HDAC have been shown to have antiproliferative and apoptotic effects in human tumor cell lines *in vitro*. Additionally, several compounds have been reported to inhibit human tumor xenograft growth in nude mice. A subset, including suberoylanilide hydroxamic acid (SAHA), FK-228 and MS-275, are under evaluation in clinical trials as anticancer agents. Our efforts to discover novel small molecule HDAC inhibitors (HDAIs) led to the identification of the potent HDAI NVP-LAQ824, which is currently in Phase I clinical trials. To support the development of NVP-LAQ824, we investigated preclinical efficacy in the athymic nude mouse, pharmacokinetics in the mouse and rat and toxicity in the rat. We report here the dose-response of NVP-LAQ824 in the HCT116 human colon, A549 human lung and MDA-MB-435 human breast xenograft models in athymic nude mice. In each of these models, tumor stasis was observed at tolerated doses and schedules. Plasma and tumor concentrations of NVP-LAQ824 were obtained from athymic mice bearing HCT116 tumors at two doses and from athymic mice bearing MDA-MB-435 tumors at one dose. These data show that NVP-LAQ824 clears rapidly from plasma and persists at significant concentrations in tumor tissue up to 16 h post-dose. An 8-cycle toxicology study in rats was carried out at three doses, followed by a 4 week recovery. Toxicity includes a reversible dose-dependent reduction in body weight gain and food consumption, and reversible changes in the myelopoi-