Retroviral Insertional Mutagenesis as a Strategy for the Identification of Genes Associated with cis-Diaminedichloroplatinum(II) Resistance

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ABSTRACT

Expression of resistance to cis-diaminedichloroplatinum(II) (CDDP), one of the most effective chemotherapeutic drugs used to treat a variety of malignancies, remains a serious obstacle for improving cancer treatment. To study possible genetic mechanisms underlying the development of CDDP resistance, we have adopted the approach of retroviral insertional mutagenesis. An early-stage CDDP-sensitive human melanoma cell line, WM35, was infected with a defective adenoviruse stem virus (murine stem cell virus), and the pooled cells were subsequently selected for CDDP-resistant variants. Nine CDDP-resistant clones independently derived from murine stem cell virus-infected WM35 cells were analyzed and it was found that five of these clones acquired an identical retroviral integration site, designated as CDDP resistance locus 1 (CRL-1), as revealed by isolation of retroviral flanking sequences. Furthermore, using the flanking sequence as probe, we have detected a 3.5–4.0-kilobase message, the expression of which is strongly increased in clones carrying a rearranged CRL-1 locus. These results strongly suggest that overexpression of CRL-1 confers resistance to CDDP in these clones. In addition, the present study indicates that retroviral insertional mutagenesis represents a potential strategy to identify genes responsible for CDDP resistance and possibly other chemotherapeutic drugs as well.

INTRODUCTION

The tendency of tumors to express resistance to anticancer chemotherapeutic drugs is one of the most significant obstacles to the successful treatment of cancer. Resistance can be either "physiological" or "cellular" (1–4). The latter mechanism refers to the ability of individual cancer cells to undergo mutations or other types of genetic alterations which, for one reason or another, biochemically renders the cells less vulnerable to the toxic effects of one or more of the given drugs. This can result from such factors as decreased drug uptake, increased drug efflux, or increased drug detoxification, among other mechanisms. Resistant cancer cells can arise in mammaries in a de novo fashion in the absence of drug selection (intrinsic drug resistance) or they can be selected for by the drug (acquired drug resistance). The discovery of genes which encode P-glycoprotein, an energy-dependent efflux pump which mediates "multidrug resistance" to structurally diverse natural lipophilic compounds, is one of the best examples of the advances made in understanding the possible genetic and biochemical mechanisms of drug resistance in cancer, at least in cell culture, and represents an excellent paradigm for the concept of cellular drug resistance (1, 3, 4). However, comparatively little is known about the nature of the genes which are involved in conferring resistance to commonly used alkylating agents and platinum drugs such as CDDP,5 which is one of the most commonly used drugs to treat a variety of malignancies, especially ovarian, testicular, and head and neck carcinomas (5).

It is widely accepted that CDDP exerts its cytotoxic effects through covalent binding to DNA to induce DNA strand breaks (single- or double-strand breaks), disturb local DNA structures, and form DNA intra- and interstrand cross-links, which interfere with DNA replication and transcription (6–8). It has, therefore, been postulated that gene(s) conferring resistance to CDDP may be located in DNA repair processes and drug detoxification (6–8). In certain CDDP-resistant cancer cells, decreased accumulation of CDDP (9–12), upregulation of glutathione or glutathione S-transferase activity (13), increased levels of metallothioneins (14, 15), and enhanced DNA-repair activity (16–19) have been correlated with the acquisition of drug resistance. However, the clinical significance of any of these mechanisms in conferring CDDP resistance has not been rigorously established (6–8). Furthermore, increases in expression of these factors are not always detected in CDDP-resistant tumor cells, suggesting that other mechanisms can be involved in acquisition of CDDP resistance.

As a means of rapidly identifying specific genes conferring resistance to CDDP, we have adopted the approach of retroviral insertional mutagenesis (proviral tagging). This approach has proved very successful in identifying oncogenes and the tumor suppressor genes in various animal tumor systems induced by slow transforming retroviruses (20, 21). Thus, using this strategy, a number of genes associated with the development of malignancy in vivo, including p53, fli-1, fli-2, and vin-1 in leukemia (22–26); myc and Tpl-1 in lymphoma (27, 28); int-1 and int-2 in mammary tumors (29, 30); as well as genes or loci involved in growth factor dependence in vitro (31, 32) have been identified. Recently, Habets et al. (33) have cloned and identified a lymphoma invasion-inducing gene, Tiam-I, by using proviral tagging in combination with in vitro selection techniques for invasion. These results indicate that proviral tagging can be used as a powerful tool to identify and clone genes involved in specific aspects of the development of malignancy, including tumor induction and progression. In theory the method should be applicable for identifying genes known to be involved in conferring to cells any selectable phenotype, including drug resistance. We decided, therefore, to infect CDDP-sensitive human tumor cells with a retrovirus containing a dominant selectable marker and then subsequently to select for CDDP-resistant variants. The rationale was that proviruses integrate randomly into the genome, and consequently a gene involved in acquisition of CDDP resistance may be mutated or activated by insertion of the provirus DNA within the gene or adjacent to it. Therefore the gene can be cloned and identified by using provirally "tagged" virus-host junction sequences as probes (34). Here we report on the selection of CDDP-resistant variants and the identification of a retroviral integration site CRL-1 (for CDDP resistance locus 1), from CDDP-resistant human mela-

5 The abbreviations used are: CDDP, cis-diaminedichloroplatinum(II); GSE, genetic suppressor elements; MSCV, murine stem cell virus; fbs, fetal bovine serum; pgk, phosphoglycerate kinase.
noma cell variants generated by retroviral infection. The flanking sequence of \textit{CRL-1} hybridizes with a 3.5-4.0-kilobase message, the expression of which is significantly increased in clones carrying the integration site, suggesting that the \textit{CRL-1} locus may be involved in conferring resistance to CDDP in these cell clones.

**MATERIALS AND METHODS**

Tumor Cell Lines and Retrovirus Infection Procedures. The WM35 human melanoma cell line (35) was originally a gift from Dr. M. Herlyn (Wistar Institute, Philadelphia, PA) to R. S. K. and was maintained in RPMI 1640 (GIBCO Laboratories, Life Technologies Inc., Grand Island, NY) supplemented with 5% heat-inactivated FBS (Hyclone Laboratories, Logan, UT). The WM35 cells were established from a patient with an early stage, curable, radial growth phase primary melanoma. Replication-defective \textit{neo}-containing MSCV with an amphotrophic host range was produced by infection of GP+envAM12 helper-free packaging cells (36) with ecotropic virus produced by GP+E+86 cells transfected with an \textit{env}-- version of MSCV (Fig. 1), as described previously (37). Viral supernatant from subconfluent cultures was collected, centrifuged, and assayed on NIH3T3 cells. Recombinant virus having a titer of 0.5-1 \times 10^6 G418-resistant colony-forming units/ml was used to infect WM35 cells. The cells were plated at a concentration of 2 \times 10^5 cells/60-mm dish and incubated at 37°C for 24 h. The MSCV viral supernatant (1.5 ml/dish) was added and incubated at 37°C for 4-8 h followed by the addition of fresh medium. After 24 h, the medium was removed and fresh medium with 800 \mu g/ml G418 (GIBCO Laboratories) was added for the selection of G418-resistant cells. One dish for each individual infection was used. The resistant cells were then expanded and used as the starting population for the isolation of CDDP-resistant variants.

Selection of CDDP-resistant Variant Cells. Cells of WM35 parental and three independent MSCV-infected WM35 cell populations (infections 1, 2, and 3) were seeded at 2 \times 10^5 cells/60-mm tissue culture dish in RPMI 1640 with 5% FBS and incubated at 37°C for 24 h. Fresh medium containing different concentrations of CDDP (6, 8, and 10 \mu M; Sigma Chemical, Co., St. Louis, MO) was added and changed every other day up to 10 days. Ten dishes for each CDDP concentration were used. The cells were then washed free of CDDP and maintained in CDDP-free medium for 3-6 weeks. Individual CDDP-resistant clones were isolated and expanded. The dishes were continuously incubated at 37°C, and cells were pooled for further analysis.

Colonies assays for CDDP-resistant Variant Cells. Cells from the WM35 parental cell line and CDDP-resistant clones (A to I) derived from WM35-infection 1 were seeded in 24-well plates at initial numbers of 10^3/well in RPMI 1640 with 5% FBS and incubated at 37°C. At different times the cells were harvested and counted using a hemocytometer. Exponentially growing cells were subjected to selection for CDDP-resistant variants. At 8 and 10 \mu M CDDP, no surviving colonies were found in the parental WM35 cell cultures. In contrast, a significant number of surviving colonies was observed in the cultures of all the infected cells 4 weeks after CDDP treatment, and all the surviving colonies were G418 resistant, suggesting that the cells harbored at least one provirus (data not shown). These results indicate that upon retroviral infection, some cells become refractory to the cytotoxic effects of CDDP and therefore acquire a selective growth advantage in the presence of CDDP contained in the medium. At the present time we do not know if the CDDP-resistant variants are cross-resistant to any other classes of chemotherapeutic drug or to other platinum analogues.

**RESULTS**

Retroviral Infection and Selection of CDDP-resistant Variants. It is well known that advanced-stage human melanomas in the clinical setting are intrinsically resistant to CDDP as well as other classes of drugs (39). Hence it could be argued that such cells would not represent a suitable cell population to use for studying acquired resistance to CDDP. However, nothing is known about the clinical CDDP sensitivity of very-early-stage melanoma cells since such patients are treated only by surgery. In preliminary experiments we found that the early-stage human melanoma cell line WM35 was very sensitive to the cytotoxic effects of CDDP in culture; the LD_{50} was <1 \mu M (data not shown). We therefore selected this cell line as a suitable target for retrovirus infection. In order to generate CDDP-resistant cells by retroviral insertional mutagenesis, the early-stage, CDDP-sensitive human melanoma cell line WM35 was infected with replication-defective, amphotropic MSCV carrying the neomycin phosphotransferase (\textit{neo}) gene. Typically, WM35 cells were first plated and grown to semiconfluence and then a 5-fold excess of MSCV retrovirus was added to the cells for 24 h. More than 80% of the virus-infected cells were found to be resistant to the neomycin analogue G418, and each cell contained one to five integrated proviruses as shown by Southern blot analysis (data not shown).

Cells from three independent infections and the parental WM35 cells were subjected to selection for CDDP-resistant variants. At 8 and 10 \mu M CDDP, no surviving colonies were found in the parental WM35 cell cultures. In contrast, a significant number of surviving colonies was observed in the cultures of all the infected cells 4 weeks after CDDP treatment, and all the surviving colonies were G418 resistant, suggesting that the cells harbored at least one provirus (data not shown). These results indicate that upon retroviral infection, some cells become refractory to the cytotoxic effects of CDDP and therefore acquire a selective growth advantage in the presence of CDDP contained in the medium. At the present time we do not know if the CDDP-resistant variants are cross-resistant to any other classes of chemotherapeutic drug or to other platinum analogues.

CDDP-resistant Profiles of Isolated Variants. Nine clones obtained from nine dishes of infection 1 cells were expanded and used to determine their \textit{in vitro} growth and relative drug resistance properties. As illustrated in Fig. 1A, no difference in intrinsic growth properties was observed among the CDDP-resistant clones, the parental WM35 cells, or the polyclonal infection 1 cells under the culture conditions tested. The resistance of these clones to the cytotoxic effects of CDDP was determined by a colony formation assay (2, 38). Clones A, E, and G from infection 1, clone 2W from infection 2, clone 3Z from infection 3 and the parental WM35 cells were exposed to CDDP for 1 h. Relative resistance (surviving fraction of colonogenic cells) to CDDP was then determined. Fig. 3 shows that clones E and G are significantly more resistant to the cytotoxic effects of different concentrations of CDDP than are the parental WM35 cells. The relative resistance ratio is about 2-fold. A similar pattern of
Resistance was obtained for clones A, 2W, and 3Z (data not shown). Furthermore, we found that CDDP resistance remains stable with serial passage in vitro. For example, cells from passages 4 and 11 of clones E and G exhibited identical levels of drug resistance. This suggests that the resistant phenotype is relatively stable and probably results from activation of genes by retroviral integration.

Identification of Viral Integration Sites in Resistant Variants. The clonality of the resistant variants derived from infection 1 was determined by analyzing their proviral integration sites. DNA isolated from these clones was digested with EcoRI, HindIII, or BamHI and subjected to Southern blot analysis using a pgk-neo probe that maps within the MCV retrovirus. Since all of the three enzymes cut only once in the viral genome (see Fig. 1), the pgk-neo probe will detect a band that represents part of the integrated provirus, and its 3'- or 5'-flanking sequences depending on the enzyme used. As shown in Fig. 4, Southern blot analysis revealed a band with an identical size in five resistant variants (clones B, D, E, F, and G) using three different restriction enzymes for the digestion. These results strongly suggest that these five clones either originated from the same cell clone or were derived from different cell clones in which viral integrations clustered in a very narrow region, a situation similar to that recently found for the locus of the invasive gene Tiam-1 activated by retroviral integration (33). Two other resistant variants (clones H and I) also showed an identical band using the three different restriction enzymes, demonstrating that they were probably derived from the same clone or different clones with a clustered viral integration. However, the origin of the H and I clones is different from those of other five cell lines because they show different rearrangement bands with the same restriction digestion. The other two cell lines (A and C) showed different bands and most likely they originated from independently infected cell clones. Lanes A-p, C-p, D-p, and E-p in Fig. 4a represent DNA isolated from pooled cells of the corresponding dish after the clone was isolated. As shown by Southern analysis, DNA in Lanes A-p, C-p, and E-p represent more than one cell clone, and interestingly, these cells are probably related to clones isolated from different dishes. For example, cells in Lane A-p are related to clone C or clones H and I; some cells in Lane C-p are related to clones B, D, E, F, and G, and some cells in Lane E-p are related to clone C or clones H and I. Frequent isolation of CDDP-resistant cell clones that acquired an identical pattern of proviral integration from a polyclonal infected cell population strongly suggests that the integrated provirus may alter the expression of a nearby cellular gene capable of conferring resistance to CDDP.

Cloning of Proviral Flanking Sequences. In order to confirm the possibility that the five clones (B, D, E, F, and G) might have originated from the same precursor or from related clones with viral integration clustered within a small region and to determine whether MCV integration activates a gene, the expression of which might confer resistance to CDDP, cloning of the viral flanking sequences was undertaken. To isolate the provirus and its flanking sequences, the pgk-neo probe was used to screen a genomic library constructed with DNA from clone E. A total of six positive clones were isolated, and plaques were purified. Phage DNA from five of these were found to contain the entire integrated MCV provirus and associated 5'- and 3'-flanking sequences, and one clone contained the 3' portion of MCV, including the pgk-neo transcriptional unit and downstream flanking sequences (Fig. 5). This viral integration site was designated CRL-1. The 6-kilobase EcoRI fragment encompassing viral pgk-neo and the 3'-flanking sequences were subcloned into the plasmid
Fig. 4. Patterns of proviral integration in CDDP-resistant variant clones. Ten μg of DNA isolated from CDDP-resistant cell variants (clones A to I) derived from infection 1, pooled WM35 cells from infection 1, and parental WM35 cells were digested with restriction enzymes EcoRI (a), HindIII (b), or BamHI (c) and hybridized with the pgk-neo probe as shown in Fig. 1. In a, Lanes A-p, B-p, C-p, D-p, and E-p are DNA isolated from pooled cells of the dish after the corresponding clone was picked up. Kbp, kilobase pairs.

Infection 1. As shown in Fig. 6, probe a detects a 4-kilobase fragment in all virally infected cell clones from infection 1 as well as from WM35 parental cells. However, this probe also detects an additional 6-kilobase rearranged fragment in DNA of clone E from which probe a was derived, as well as in the four other clones B, D, F, and G. The identical size of the rearranged fragment in these clones is in agreement with the results obtained with pgk-neo probe and again demonstrates their clonal origin. No rearrangement of the CRL-1 locus was detected in other CDDP-resistant cell lines (clones A, C, H, and I).

Identification of CRL-1 Transcripts. To address whether the integration of MSCV within the CRL-1 locus resulted in altered expression of a transcription domain responsible for CDDP resistance, RNA isolated from clones with or without the CRL-1 rearrangement and from parental WM35 cells were separated on a formaldehyde gel and hybridized with probes a, c, or d derived from plasmid pE6B (Fig. 5). Neither probe a nor d hybridized with any specific transcripts. However, as shown in Fig. 7, probe c detected a 3.5-4.0-kilobase transcript, the expression of which significantly increased in clones E and G, both of which carried a rearranged CRL-1 locus. Three other CRL-1 rearrangement-positive clones (B, D, and F) also expressed a high level of this message (data not shown). The expression of the CRL-1 message in CDDP-resistant clones A and C and in parental pGEM(7Z), and the pgk-neo cassette was removed by BamHI digestion to generate plasmid pE6B. A 1.1-kilobase BstXI fragment isolated from plasmid pE6B (probe a in Fig. 5) appeared to be free of repetitive sequences and was used to hybridize a Southern blot containing DNA from the nine CDDP-resistant variants derived from infection 1. As shown in Fig. 6, probe a detects a 4-kilobase fragment in all virally infected cell clones from infection 1 as well as from WM35 parental cells. However, this probe also detects an additional 6-kilobase rearranged fragment in DNA of clone E from which probe a was derived, as well as in the four other clones B, D, F, and G. The identical size of the rearranged fragment in these clones is in agreement with the results obtained with pgk-neo probe and again demonstrates their clonal origin. No rearrangement of the CRL-1 locus was detected in other CDDP-resistant cell lines (clones A, C, H, and I).

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DISCUSSION

Mechanisms of drug resistance in cancer have been generally investigated through selection of resistant variants by continuous or intermittent exposure of cells to chemotherapeutic drugs in an incremental fashion, usually over prolonged periods. Established resistant variants are then subjected to genetic analysis by means of either a protein-antibody approach or mRNA subtractive hybridization. Such approaches have been found to be very fruitful for isolating genes conferring multidrug resistance to tumor cells in culture; the cloning and identification of mdr and mrp genes are two examples of the success of this strategy (40, 41). However, attempts to obtain relatively stable resistant variants and then to identify the genes related to the phenotypes, these approaches have been very time consuming, usually taking several years. Recently, an expression cloning strategy has been developed for the identification of drug resistance genes with the combination of a stepwise selection of resistant variants, revealing the mrp gene as a multidrug-resistant gene in certain cell lines (42).

Once again, however, expression cloning cannot avoid the time consuming process of selection of resistant variants. In a totally different approach, Gudkov et al. (43, 44) adopted the strategy of GSE to identify genes the down-regulation of which confers resistance to certain chemotherapeutic drugs in cells grown in culture. The GSE method has confirmed topoisomerase II as a target for some alkylating agents (43) and identified several novel candidate genes, such as the one encoding kinesin, the alteration of which might be related to the resistance of a few hundred base pairs of the CRL-1 locus, size differences of rearranged fragments by Southern blot analysis with two probes, either pgk-neo or genomic fragment probe a. This suggests that these five clones are derived from the same precursor cell that clonally expanded following infection with MCV. Since MCV integration at the CRL-1 locus activates a transcriptional unit, the expression of which appears to confer resistance to CDDP. Cells originating from the same precursor would acquire growth and survival advantages in medium containing high concentrations of CDDP and are thus preferentially selected for during the isolation of CDDP-resistant variants. Alternatively, it is possible that these five clones originated from different but related precursors. The MCV viruses could integrate in a narrow region around the CRL-1 locus and activate the same gene. All the clones, therefore, show similar resistance profiles against CDDP and are equally, but also preferentially, selected for after exposure to high concentrations of CDDP. Since viral integrations are clustered within a few hundred base pairs of the CRL-1 locus, size differences of rearranged alleles cannot be distinguished by Southern analysis. This assumption, however, must be verified by sequencing the viral integration sites in all clones.

Habets et al. (33) have recently demonstrated two provirus integration clusters within the tumor-invasive gene designated Tiam-1 (33). In each cluster, proviruses integrated in the same transcriptional orientation within a few hundred base pairs and showed fragments of rearrangement of similar size in Southern blots (33). Nevertheless, expression of which results in the acquisition of drug resistance, and the latter mechanism of drug resistance may account for a substantial proportion of clinical cases of acquired drug resistance.

Our present study demonstrates the application of retroviral insertional mutagenesis, which has been successfully used to identify genes related to tumor invasion (33) and antioestrogen resistance (31), for the possible identification of CDDP resistance genes. Thus we have shown that WM35 human melanoma cells infected with the retrovirus MCV are more resistant to CDDP than are parental WM35 cells. It is worth noting that the resistance level in clones obtained by retroviral infection of this study is about 2-fold compared to the parental WM35 cells, which is not as high as is generally obtained using long-term classically in vitro selected resistant variants. Nevertheless, this level of resistance is comparable to that observed with respect to clinical levels of acquired drug resistance and can be responsible for converting responders to nonresponders in the context of cancer chemotherapy (45–48).

Using both viral and integration site-specific probes isolated from a CDDP-resistant clone, we have identified an integration site, CRL-1, that occurs in five of nine resistant variants independently derived from infection 1. Surprisingly, all five clones show identical rearranged fragments by Southern blot analysis with two probes, either pgk-neo or genomic fragment probe a. This suggests that these five clones are derived from the same precursor cell that clonally expanded following infection with MCV. Since MCV integration at the CRL-1 locus activates a transcriptional unit, the expression of which appears to confer resistance to CDDP. Cells originating from the same precursor would acquire growth and survival advantages in medium containing high concentrations of CDDP and are thus preferentially selected for during the isolation of CDDP-resistant variants. Alternatively, it is possible that these five clones originated from different but related precursors. The MCV viruses could integrate in a narrow region around the CRL-1 locus and activate the same gene. All the clones, therefore, show similar resistance profiles against CDDP and are equally, but also preferentially, selected for after exposure to high concentrations of CDDP. Since viral integrations are clustered within a few hundred base pairs of the CRL-1 locus, size differences of rearranged alleles cannot be distinguished by Southern analysis. This assumption, however, must be verified by sequencing the viral integration sites in all clones.

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because retrovirus integration in the genome is believed to take place in a relatively random manner (49), the occurrence of CRL-1 in five of nine CDDP resistant clones strongly suggests that viral integration within the CRL-1 locus is tightly linked to the selected biological phenotype of CDDP resistance. In our study, by using integration-specific sequences as a probe, we have demonstrated that MSCV integrated in the CRL-1 locus activates a gene with a transcript of 3.5—4.0 kilobases and that the increase in expression of this gene may account for the acquisition of resistance to CDDP. In a recent study, Lepage et al. (50) have found that two intrinsically multidrug-resistant mouse lymphoid tumor cell lines, P388/VCR-2 and P388/DAM-2, contain a rearranged mdr3 gene. Detailed analysis reveals that mdr3 gene rearrangements in P388/VCR-2 and P388/DAM-2 resulted from integrations of mouse mammary tumor virus and intracisternal A particle, respectively. Integration of either mouse mammary tumor virus or intracisternal A particle in the mdr3 locus leads to the overexpression of the mdr3 gene and confers multidrug resistance in these cells (50). These findings and our results indicate that gene tagging by viral insertional mutagenesis in vitro can be an effectively alternative way to identify and clone genes related to drug resistance.

The basis for resistance to CDDP, both in vitro and in vivo, remains largely undefined, although a number of studies have indicated that multiple mechanisms are likely involved (6—8). Enhanced DNA repair as a mechanism of resistance to CDDP has been observed in vitro-induced cell lines and resistant tumor cells obtained from patients after treatment with CDDP (11, 16—18). This enhanced repair process is accompanied by either increased amounts of repair enzymes (51, 52) or DNA-binding proteins specifically recognizing damaged DNA (53—57). Another mechanism of CDDP resistance is reduced accumulation of CDDP in resistant cells (9, 10). Many studies have implicated a CDDP accumulation defect as an important way to acquire CDDP resistance in both murine and human carcinoma cell lines (12, 58, 59). Furthermore, reduced platinum accumulation has recently been associated with specific changes in plasma membrane proteins (59, 60) and the function of Na+,K+-ATPase (61, 62) in resistant cells. Other biochemical changes related to CDDP resistance include increased levels of metallothioneins (14, 15), glutathione, and elevated activity of glutathione S-transferase (13). In our present study, virus integration at the CRL-1 locus activates a gene with a message of 3.5—4.0 kilobases, which is different in size from those genes encoding any of the above factors. Transfer of this sequence into CDDP-sensitive WM35 cells using appropriate gene transfer expression vectors should reveal direct evidence of its role in conferring resistance to CDDP and possibly other drugs as well.

In summary, using retroviral insertional mutagenesis we have succeeded in inducing resistance to CDDP in WM35 human melanoma cells which are otherwise highly sensitive to this drug. The analysis of viral integrations led to the identification of a locus designated CRL-1 the expression of which is activated by the integrated MSCV. This observation strongly suggests that this approach can be used as a strategy for the identification of a gene family or a group of genes involved in resistance to CDDP. Furthermore, our results raise the possibility that a similar selection system could also be applicable for isolation of genes associated with acquisition of resistance to other classes of chemotherapeutic drugs in different types of cancer.

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REFERENCES

Retroviral Insertional Mutagenesis, Drug Resistance


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