A Mutation in Aminopeptidase N (CD13) Isolated from a Patient Suffering from Leukemia Leads to an Arrest in the Endoplasmic Reticulum*

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Human aminopeptidase N (APN) is used as a routine marker for myelomonocytic cells in hematopoietic malignant disorders. Its gene and surface expressions are increased in cases of malignant transformation, inflammation, or T cell activation, whereas normal B and resting T cells lack detectable APN protein expression. In this study we elucidated the intracellular distribution, expression pattern, and enzymatic activity of a naturally occurring mutation in the coding region of the APN gene. At physiological temperatures the mutant protein is enzymatically inactive, persists as a mannose-rich polypeptide in the endoplasmic reticulum, and is ultimately degraded by an endoplasmic reticulum-associated degradation pathway. It shows in part the distinct behavior of a temperature-sensitive mutant with a permissive temperature of 32 °C, leading to correct sorting of the Golgi compartment accompanied by the acquisition of proper glycosylation but without reaching the cell-surface membrane and without regaining its enzymatic activity. Because the patient bearing this mutation suffered from leukemia, possible links to the pathogenesis of leukemia are discussed.

Membrane alanyl aminopeptidase (aminopeptidase N, APN2) is a 967-amino acid type II transmembrane protein that is expressed on the surface of a broad variety of cell types, most strongly in intestinal mucosa and kidney tissue (1, 2). Although not all aspects of its function are fully understood, it is established that the enzyme preferentially cleaves neutral amino acids from the N terminus of oligopeptides leading to degradation of neuropeptides (3–12), cytokines, immunomodulatory peptides (13–15), and angiotensins (16–18). Depending on its location, APN is involved in terminal degradation of small peptides in the intestinal brush border (19), inactivation of endorphins and enkephalins in synaptic membranes (2), and angiogenesis (21–23).

Furthermore, APN may contribute to extracellular matrix degradation (24, 25) and antigen processing via trimming of major histocompatibility complex class I and II associated peptides (26, 27). There are also several reports of its distinct functions as a receptor for various viruses like the human and murine cytomegalovirus (28–30) or different coronaviruses (31–34). Inhibitors of APN have been shown to inhibit the growth of tumor cells in a mouse model (35–37), as well as gastrointestinal tumors and T cell leukemia cells in man (38–40). Recently it has been shown that APN is capable of promoting phagocytosis by supporting Fc-γ receptors on peripheral blood monocytes (41).

APN has been implicated in the growth and function of immune cells, including T cells and T cell subsets (42–44). A significant fraction of malignant lymphocytes and corresponding cell lines appear CD13-positive in flow cytometry. In addition, CD13-negative T and B cell lines were shown to contain considerable amounts of APN mRNA (45). Therefore, it is suggested that a dysregulation of APN expression may contribute to or result from malignant transformation of lymphocytes and/or enhanced cellular growth. A previous study reported mutations in the gene coding for APN in 18 and 6% of cases of leukemia and lymphoma, respectively. No such mutations were found among healthy controls (46).

We show here that cloning and sequencing of mutant APN-DNA fragments from a patient with lymphoma revealed the presence of a silent mutation not expected to affect protein structure and function and the substitution (Leu-243 → Pro) of a single amino acid residue. Further analysis of the latter mutation revealed significant effects on APN expression and enzymatic activity.

EXPERIMENTAL PROCEDURES

Materials and Reagents—Streptomycin, penicillin, glutamine, Dulbecco’s modified Eagle’s medium, methionine-free Dulbecco’s modified Eagle’s medium, fetal calf serum, and trypsin were purchased from BioWest, Essen, Germany. Pepstatin, leupeptin, aprotinin, trypsin inhibitor, and molecular mass standards for SDS-PAGE were purchased from Sigma. Soybean trypsin inhibitor was obtained from Roche Diagnostics. L-[^35S]Methionine (1000 Ci/mmoll) and protein A-Sepharose were obtained from Amersham Biosciences. Acrylamide, N,N’-methylenebisacrylamide, and TEMED were purchased from Carl Roth GmbH, Karlsruhe, Germany. SDS, ammonium persulfate, dithiothreitol, and Triton X-100 were obtained from Merck.

pEGFP-N1 vector, pDsRed2-ER, and pECPF-Golgii were purchased from Clontech. pDsRed-Golgii vector was constructed by subcloning dsRed from pEDsRed1-N1 (Clontech) into pECPF-Golgii with BamHI and NotI. Restriction enzymes were obtained from MBI Fermentas, St. Leon-Rot, Germany. Isis polymerase was obtained from Qbiogene, Heidelberg, Germany.
**Immunocchemical Reagents**—For immunoprecipitation of human APN, tagged with GFP, the GFP-antibody, purchased from Clontech was used.

**Construction of cDNA Clones**—First, wild type human APN from U937 cells was cloned into the pEGFP-N1 vector, resulting in the expression vector pEGFP-wt-APN containing enhanced GFP fused in-frame to the 3’ end of wild type APN. Second, the plasmid pEGFP-APN_{L243P} was generated with the QuickChange™ in vitro mutagenesis system from Stratagene, according to the manufacturer’s instructions, by using the pEGFP-APN (wild type) as the template.

The following mutagenesis oligonucleotide (both sense and antisense) was used, 5’- TGTCCAACATG-3’

APNL243P was generated with the QuickChange™ system from Stratagene, according to the manufacturer’s instructions, by using the pEGFP-APN (wild type) as the template.

**Transient Transfection of COS-1 Cells, Biosynthetic Labeling, and Immunoprecipitation**—COS-1 cells were transiently transfected with DNA by using DEAE-dextran essentially as described previously (47). The plasmids pEGFP-APN and pEGFP-APN_{L243P} were used throughout the studies. 48 h after transfection, the cells were biosynthetically labeled. The cells were incubated in methionine-free minimum Eagle’s medium containing 50 µCi of [35S]methionine for the indicated time intervals and chased in pulse-chase experiments with nonlabeled methionine for different periods of time. Subsequently the cells were rinsed twice with ice-cold phosphate-buffered saline and solubilized with 1 ml of lysis buffer containing 25 mM Tris, 150 mM NaCl, 0.5% deoxycholate, and 0.5% Triton X-100, supplemented with 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstatin, 5 µg/ml leupeptin, 5 µg/ml aprotinin, and 50 µg/ml trypsin inhibitor for 30 min at 4 °C. After 1 h of preclearing with 30 µl of protein A-Sepharose, the immunoprecipitation was performed with the anti-GFP mAb and 50 µl of protein A-Sepharose as described previously (47). The immunoprecipitates were further processed by SDS-PAGE according to Laemmli. After electrophoresis, the gels were fixed and analyzed by a phosphorimaging device (Bio-Rad). In another set of experiments in which the turnover kinetics and degradation of the mutant were analyzed, the transfected cells were pulsed for 1 h with [35S]methionine and chased for 24 h. The labeling intensities of the mutant APNL243P were estimated. Equal amounts of radioactivity were used in the immunoprecipitations. The labeling intensities of the mutant APNL243P were assessed relative to the first chase time point.

**Cell Surface Immunoprecipitation**—Wild type and mutant APN antigens were isolated from the surface of COS-1 cells as described previously (49). Transiently transfected COS-1 cells were labeled with 50 µCi of [35S]methionine for 4 h at 37 °C, 12 h at 20 °C, or 8 h at 32 °C. Subsequently the cells were shifted to 4 °C, and wild type or mutant APN expressed at the cell surface was labeled by mAb anti-GFP added to the medium for 1 h. The cells were solubilized in lysis buffer, and APN-GFP was immunoprecipitated with protein A-Sepharose prior to SDS-PAGE analysis.

**Trypsin Treatment of Cell Lysates**—The immunoprecipitates from transiently transfected, [35S]methionine-labeled COS-1 cells were treated with 0, 2, 4, 6, 8, or 10 µg/µl trypsin for 30 min at 37 °C. The reaction was terminated by cooling on ice at 4 °C and addition of 200 µg of soybean trypsin inhibitor (Roche Diagnostics).

**Confocal Fluorescence Microscopy**—Confocal images of living cells were acquired 2 days after transfection on a Leica TCS SP2 microscope with a ×63 water planapochromat lens (Leica Microsystems). Dual color GFP and dsRed images were obtained by sequential scans with the 468-nm excitation line of an argon laser or the 543-nm excitation line of a He/Ne laser, respectively, and the optimal emission wavelength for GFP or dsRed, as described previously.

**Measurement of APN Enzymatic Activity**—Cells were resuspended in PBS and solubilized by sonication on ice for 30 min. The suspension was centrifuged at 60,000 × g for 30 min, and the particulate fraction was resuspended in 500 µl of PBS. GPF fluorescence was read at 488/507 nm using the luminescence spectrophotometer LS50 (PerkinElmer Life Sciences) and used to normalize for APN activity. The activity of APNL243P was determined by measuring the hydrolysis of Ala-p-nitroanilide (50) ratio proportional to wt-APN.

**Transient Transfection of N109 Cells, Invasion, and Proliferation Assays**—The renal carcinoma cell line N109 was recently established from a bone metastasis of a typical clear cell renal carcinoma at the Institute of Pathology, University of Magdeburg, Germany. It does not express any APN mRNA or protein. N109 cells were transiently transfected with wt-APN or APNL_{L243P} DNA by using Effectene (Qiagen) as described previously (47). Proliferation of N109 cells transfected with either wt-APN or APNL_{L243P} in comparison to sham-transfected cells was determined by measuring [3H]thymidine incorporation as described (44). Cellular invasion through a Matrigel-coated 24-well transwell chamber (Costar) was analyzed as described recently (51). Briefly, 48 h after transfection, N109 cells were incubated for another 24 h in the invasion chambers. Cells passing the membrane and attaching to the lower side of the Matrigel-coated membranes (invasive cells) were harvested using trypsin/EDTA, and the cell number was assessed using a Coulter Counter ZII.

**RESULTS**

The mRNA of peripheral blood mononuclear cells of a male 67-year-old patient suffering from non-Hodgkin lymphoma (centroblastic, IIIb) was isolated and transcribed into cDNA as described (52). Amplified DNA was sequenced by SSCP analysis, and two mutations were identified. Besides a silent mutation at position 115, an exchange from T to C at position 728 leads to replacement of leucine by proline at amino acid 243. The complete absence of T at position 728 in the sequences obtained indicate the homozgyotic nature of this mutation (Fig. 1). This mutant was termed APNL_{L243P} and subsequently cloned into a pEGFP-N1 vector.

Biochemical studies of APNL_{L243P} showed that it was predominantly expressed as an endo H-sensitive mannose-rich polypeptide (Fig. 2,
**A Mutation of APN Causes Arrest in the ER**

APN₃, whereas the wt-APN shows the typical pattern of a correctly processed glycoprotein, consisting of the mannose-rich precursor form (APN₃-, ~170 kDa) and a complex glycosylated mature form (APN₃, ~190 kDa, consisting of the 166-kDa high monomeric form of mature APN enlarged by 27 kDa of monomeric GFP) (Fig. 2). Treatment with endo H led to a shift of the mannose-rich band down to ~140 kDa in both cases, whereas the band representing the complex glycosylated wt-APN did not change (Fig. 2). Fig. 2 also shows that another mutant, APNΔ227P, which has been detected previously in a patient suffering from non-Hodgkin lymphoma (46) and is included for comparison, is expressed also as an endo H-sensitive mannose-rich polypeptide.

Pulse-chase experiments of mutant and wild type APN showed that mutant APN₁₂₄₃P persisted as a mannose-rich species throughout the entire chase periods. By contrast, the wild type counterpart acquired complex glycosylation and endo H resistance already after 2 h of chase (Fig. 3).

From these data we conclude that the mutant protein is not able to mature in the Golgi to a complex glycosylated protein because of impaired trafficking and intracellular accumulation in the ER.

To explore this further, confocal microscopy analysis was used to visualize the subcellular location of APN₁₂₄₃P. The mutant protein showed a distinct pattern of staining strongly associated with the ER (Fig. 4). In contrast, wt-APN was located in Golgi vesicles as well as on the cell-surface membrane, indicating that C-terminal attachment of GFP to APN does not change its normal subcellular localization (nor its function as shown by enzymatic analyses; data not shown).

To confirm the intracellular localization, we co-transfected wt-APN and the L.243P mutant with either pDsRed-ER, containing the signal sequence of calreticulin, a specific marker for the ER, and a C-terminal KDEL ER-retention sequence, or a Golgi-specific marker, the N-terminal 81 amino acids of human β1,4-galactosyltransferase (GT) tagged with dsRed. As shown in Fig. 4, wild type APN co-localized with both the ER and Golgi markers and furthermore was seen on the cell-surface membrane, whereas the mutated protein showed a distribution pattern identical to the ER marker only, with no structures beyond the ER being stained. By having assessed the intracellular localization of the APN₁₂₄₃P mutant in the ER as a mannose-rich polypeptide, we wanted next to determine the turnover rate and the fate of this mutant. For this, a pulse-chase experiment was performed employing prolonged chase time points. As shown in Fig. 5A, the mutant started to disappear steadily throughout the chase time points and was barely detectable after 36 h of chase. By contrast, wt-APN was still persistent, predominantly in its complex glycosylated form at the same time point (Fig. 5B). This finding indicates that the mutant is intracellularly degraded, and its turnover is substantially higher than that of the wild type protein. We therefore addressed the possible degradation of APN₁₂₄₃P by an ER-associated degradation mechanism, and we analyzed the mutant and its wild type counterpart in a pulse-chase experiment using lactacystin, an inhibitor of the proteasome function. The proportions of the mutant protein during the various chase time points were compared with that at the initial chase time point. As shown in Fig. 5A, a dramatic shift in the stability of the mutant is observed in the presence of the inhibitor. Almost 85% of the mutant persisted after degradation at 12 h of chase as compared with 40%
without the inhibitor, and after 36 h of chase ~65% was still present as compared with 25% without the inhibitor. This result is compatible with a proteasome-mediated degradation of the mutant.

Many mutant phenotypes of membrane proteins in human diseases reveal the characteristics of temperature-sensitive mutants that acquire partial folding at the permissive temperature and subsequently are able to exit the ER to the Golgi and ultimately to the cell surface (52). At the nonpermissive temperature, these mutants undergo degradation in their initial mannose-rich polypeptides.

To assess whether the APNL243P is temperature-sensitive, we analyzed its trafficking at various temperatures. Here, transiently transfected COS-1 cells were cultured at 22, 28, or 32 °C for 24 h prior to biosynthetic labeling and immunoprecipitation. In the case of the 22 and 28 °C incubations, no change in the glycosylation pattern of the mutant protein was observed, whereas at 32 °C APNL243P showed a noticeable complex glycosylation pattern, suggesting that at this temperature the protein can acquire at least a partial transport competence.

A pulse-chase experiment with a 30-min labeling period followed by a chase of up to 24 h showed that at 32 °C APNL243P gained complex glycosylation after 4 h, which is identical to the wt-APN (Fig. 6). But in contrast to wt-APN, the band representing the mannose-rich form of APNL243P did not disappear after 8 h but was only slightly faded after 16 h. In addition to conversion to the complex glycosylated form, this could be also due to general protein degradation.

Next we investigated if APNL243P is able to reach the cell-surface membrane by performing a cell-surface immunoprecipitation at 32 °C (Fig. 7). A complex glycosylated form of wt-APN was clearly detectable on the cell surface at 32 °C, whereas in the case of APNL243P, no protein was found on the cell membrane either directly at 32 °C or after a chase of 1 h at 37 °C following the 32 °C incubation period. In the total lysate, the mannose-rich form as well as the complex glycosylated form was present, indicating that the mutant is able to reach the Golgi but not the cell surface.
A Mutation of APN Causes Arrest in the ER

FIGURE 9. Enzyme activity. Cells were transiently transfected with the cDNAs of wt-APN or APNL243P. After 48 h, cells were scrubbed in PBS and homogenized by sonication on ice for 30 min. The suspension was centrifuged at 60,000 × g for 30 min, and the particulate fraction was resuspended in 500 μl of PBS, and the activity of APN was measured using Alap-nitroanilide as substrate. APN wild type activity was defined as 100%, and untransfected cells were used as a control. For determination of temperature susceptibility, a shift to 32 °C was performed 12 h before processing.

FIGURE 10. Trypsin treatment. COS-1 cells were transiently transfected with the cDNAs of wt-APN or APNL243P and biosynthetically labeled with [35S]methionine for 6 h. Homogenates of these specimens were immunoprecipitated with mAb anti-GFP. Immunoprecipitates were subjected to a trypsin digestion for 30 min at 37 °C at the indicated concentrations prior to SDS-PAGE analysis on 6% slab gels. Gels were analyzed by fluorography.

chemical findings are strongly supported by our analysis of the subcellular distribution of APNL243P in COS-1 cells at 32 °C by confocal microscopy. In some cells the mutant protein was present in distinct vesicles clearly associated with the cis-Golgi network, as proven by co-localization with pDsRed-Golgi. No molecules were found at the cell-surface membrane (Fig. 8). However, APN was not located in all cells in the Golgi, indicating that progression of the mutant protein to the Golgi apparatus remains somehow restricted. wt-APN used as a control appeared as fully transport competent at 32 °C as at 37 °C (data not shown).

Analysis of protein enzymatic activity revealed that APNL243P is enzymatically inactive at 32 °C as well as at physiological temperatures, whereas wt-APN exhibits a definite activity at both temperatures (Fig. 9). From these results we conclude that the mutated protein APNL243P, although partially transport competent at 32 °C, is not capable of reaching the cell-surface membrane nor of regaining enzymatic activity.

Finally we analyzed the folding status of the APN mutant. Immunoprecipitated protein was treated with trypsin at different concentrations (Fig. 10). wt-APN was only marginally degraded by trypsin even over long periods of time and increased concentrations of trypsin, indicating a strong resistance toward this protease. In contrast, APNL243P was degraded very quickly already at low trypsin concentrations. Presumably, the abnormal folding of the APN mutant leads to the exposure of potential trypsin-cleavage sides, resulting in an easily degradable protein. In the case of APNL243P, the trypsin digestion pattern did not change when the cells were incubated at 32 °C for 24 h prior to immunoprecipitation, indicating a similar folding pattern at 32 °C compared with that at physiological temperature (data not shown).

To assess any functional consequences resulting from the APNL243P mutation, we transfected the fully APN-negative N109 cell line with both wt-APN and APNL243P and determined cellular proliferation and invasion through a Matrigel-coated membrane. As observed for COS-1 cells, transfection with wt-APN resulted in an expression at the cell surface of APN, whereas the L243P mutant was retained intracellularly (data not shown) (Fig. 11A). Expression of wt-APN in N109 cells increased cellular proliferation when compared with sham-transfected cells. In contrast, N109 cells transfected with APNL243P showed no such increase in proliferation (Fig. 11B). Similarly, cellular invasion of N109 cells increased after transfection with APN wt but not after transfection with APNL243P (Fig. 11C).

DISCUSSION

The homozygotic mutation L243P characterized in a patient suffering from leukemia elicits substantial changes in the biosynthetic features, intracellular transport, and biological function of the membrane glycoprotein APN. Thus, altered folding of the mutant APNL243P protein leads to its accumulation in the ER as an inactive protein that is ultimately degraded by an ER-associated degradation pathway. Retention of proteins in the ER is known to be linked to several severe diseases like Gaucher disease (53), cystic fibrosis (54) or congenital muscular dystrophies (55). Interestingly, APNL243P reveals characteristics of a temperature-sensitive mutant. In fact, APNL243P acquires partial folding at a permissive temperature of 32 °C that results in transport competence from the ER to the Golgi and acquisition of complex glycosylation. The enzymatic function of APNL243P is, however, not restored under these conditions, suggesting different folding determinants for trafficking and function within the APN molecule. These observations clearly indicate that by exchange of a single leucine to proline, the minimal folding requirements of the mutated APN protein are no longer maintained, resulting in a misfolded protein that lacks the competence to pass from the ER into the Golgi apparatus under physiological conditions. Proline is known to have a strong helix-breaking ability, which is why we expect that its introduction into the mutant L243P causes a change in the protein conformation associated with a loss of function. Incubation at a decreased temperature of 32 °C results in a partial transport competence but not in a significant difference in folding, as could be concluded from the unchanged resistance toward trypsin. Thus we propose that the transport is more a failure of ER exit control mechanisms at lower temperatures than a significant conformational change in the APN molecule itself.

Temperature-sensitive mutants of glycoproteins are well known and were described with a wide spread of the nonpermissive temperature (56–59), but the described mutant is to our knowledge the first naturally occurring mutation affecting protein expression, folding and function,
A Mutation of APN Causes Arrest in the ER

Here, we investigated the effect on proliferation and invasion, two key features of malignant cells, of introducing APNwt or APNL243P into a fully APN-negative cell line, N109. The absence of any APN-mRNA expression distinguishes this cell line from different hematopoietic cell lines that have been analyzed previously for APN expression (45). Our results demonstrate that increased expression of wtAPN is associated with enhanced cellular proliferation and invasion and, thus, are in line with previous reports showing growth inhibitory effects in response to an inhibition of APN enzymatic activity (42). Therefore, it should be concluded that it is neither proliferation nor invasion whereby down-regulation (mutation) of APN could primarily contribute to a malignant phenotype. However, the development, growth, and spreading of tumor cells does involve a multitude of other molecular and cellular mechanisms, which still might gain profit from compromised APN expression/activity. Changes in intracellular signaling pathways and, in particular, the induction of a pro-angiogenic expression profile are among the mechanisms that warrant further investigation.

In summary, it could be conceived that changes in glycosylation, folding, or cellular localization of APN, in addition to the lack of enzymatic activity, alter the susceptibility of a cell to malignant transformation. However, the detailed mechanism needs to be addressed in future studies. Nevertheless, as far as we know, the reported mutant could be the first direct link between a mutation in the mRNA coding for APN and leukemia.

observed in the human APN gene. As it has been isolated from a patient suffering from leukemia, it is tempting to speculate that it could somehow contribute directly or indirectly to the disease.

Normal myeloid cells of monocytic and granulocytic origin express the metallopeptidase cluster of differentiation 13 (CD13) on the surface just as leukemic blasts in most acute myeloid leukemias (60–62). Because of activation or malignant transformation, other leukocyte subpopulations including human B and T cells acquire significant APN gene and surface expression. Abnormal expression of “myeloid” APN on malignant lymphocytes, the activation-dependent induction of APN surface expression on tumor-infiltrating and chronic inflammatory site peripheral T cells, and the strong anti-proliferative effects of aminopeptidase inhibitors point to a linkage of APN expression and/or function to leukocyte growth (20, 63).

This view is supported by the observation that mutations in the gene coding for APN have been found in a number of patients suffering from leukemia and lymphoma, although these mutations are rare or absent among healthy controls (51). The molecular mechanisms by which dysregulation of APN expression/activity might contribute to the malignant phenotype remain to be elucidated. An important aspect in this context is to understand the consequences that a given mutation exerts on specific enzymatic activity, cellular localization, and trafficking. Here we show that the L243P mutation, originally described in a patient suffering from non-Hodgkin lymphoma, severely compromises activity, cellular localization, and trafficking, glycosylation, and the structure of APN. Thus, the lymphoma-derived mutation L243P is indeed associated with impaired APN function, which supports the idea that dysregulation of APN contributes to tumor development or progression. APN is involved in growth, differentiation, proliferation, and effector functions of myeloid cells and lymphocytes.

REFERENCES
1. Barnes, K., Walkden, B. J., Wilkinson, T. C., and Turner, A. J. (1997) J. Neurochem. 68, 570–577
2. Lucius, R., Sievers, J., and Mentlein, R. (1995) J. Neurochem. 64, 1841–1847
3. Ahmad, S., Wang, L., and Ward, P. E. (1992) J. Pharmacol. Exp. Ther. 260, 1257–1261
4. Furushashi, M., Mizutani, S., Kurachi, O., Kasugai, M., Narita, O., and Tomoda, Y. (1988) M. Exp. Clin. Endocrinol. 92, 235–237
5. Giros, B., Gros, C., Solhonne, B., and Schwartz, J. C. (1986) Mol. Pharmacol. 29, 281–287
6. Miller, B. C., Ackroyd, A., Hersh, L. B., and Gottam, G. L. (1994) Regul. Pept. 50, 87–98
7. Miller, B. C., Thiele, D. L., Hersh, L. B., and Gottam, G. L. (1994) Arch. Biochem. Biophys. 311, 174–179
8. Mizutani, S., Goto, K., Nomiura, S., Ino, K., Goto, S., Kikawa, F., Kurachi, O., Goldstein, G., and Tomoda, Y. (1993) Res. Commun. Chem. Pathol. Pharmacol. 82, 65–80
9. Shibanoki, S., Weinberger, S. B., Ishikawa, K., and Martinez, J. L., Jr. (1991) Regul. Pept. 32, 267–278
10. Shimamura, M., Hazato, T., and Iwaguchi, T. (1988) Brain Res. 445, 350–353
11. Shimamura, M., Hazato, T., and Iwaguchi, T. (1991) J. Biochem. (Tokyo) 109, 492–497
12. Ward, P. E., Benter, I. F., Dick, L., and Wilk, S. (1990) Biochem. Pharmacol. 40, 1725–1732
A Mutation of APN Causes Arrest in the ER

13. Hoffmann, T., Faust, J., Neubert, K., and Ansorge, S. (1993) *FEBS Lett.* **366**, 61–64
14. Kanayama, N., Kajiwara, Y., Goto, J., el Maradny, E., Maehara, K., Andou, K., and Terao, T. (1995) *J. Biol. Chem.* **270**, 129–134
15. Mathe, G. (1987) *Cancer Detect. Prev.* **1**, (suppl.) 445–455
16. Chandel, D., Cepek, S., Vandermeersch, S., Ruffet, E., Fournie-Zaluski, M. C., and Ardaillou, R. (1998) *Ann. J. Physiol.* **275**, F535–F542
17. Palmeiri, F. E., Baushack, H. H., and Ward, P. E. (1989) *Biochem. Pharmacol.* **38**, 173–180
18. Palmieri, F. E., Petrelli, J. J., and Ward, P. E. (1985) *Biochem. Pharmacol.* **34**, 2309–2317
19. Nomura, R., Kiyota, A., Suzaki, E., Kataoka, K., Ohe, Y., Miyamoto, K., Senda, T., and Delmas, B., Gelfi, J., Sjostrom, H., Noren, O., and Laude, H. (1993) *Biochem. Pharmacol.* **46**, 107–113
20. Sakuraya, M., Tamura, J., Itoh, K., Kubota, K., and Naruse, T. (2000) *Int. J. Cancer* **89**, 214–221
21. Bauvois, B. (2004) *OncoGene* **23**, 317–329
22. Sato, Y. (2004) *Blood* **103**, 214–221
23. Bhagwat, S. V., Lahdenranta, J., Giordano, R., Arap, W., Pasqualini, R., and Shapiro, L. H. (2001) *Blood* **97**, 652–659
24. Fujii, H., Nakajima, M., Saiki, I., Yoneda, J., Azuma, I., and Tsuruo, T. (1995) *Clin. Exp. Metastasis* **13**, 337–344
25. Saiki, I., Fujii, H., Yoneda, J., Abe, F., Nakajima, M., Tsuruo, T., and Azuma, I. (1993) *Int. J. Cancer* **54**, 137–143
26. Larsen, S. L., Pedersen, L. O., Buus, S., and Stryhn, A. (1996) *Exp. Med. Biol.* **184**, 183–189
27. Dong, X., An, B., Salvucci, L., Storkus, W., Amosato, C., and Salter, R. (2000) *J. Immunol.* **164**, 129–135
28. Kasman, L. M. (2005) *Virology* **334**, 1–9
29. Soderberg, C., Giugni, T. D., Zaia, J. A., Larsson, S., Wahlberg, J. M., and Moller, E. (1993) *J. Virol.* **67**, 6576–6585
30. Larsson, S., Soderberg-Naucler, C., and Moller, E. (1998) *Transplantation* **66**, 411–415
31. Teager, C. L., Ashmun, R. A., Williams, R. K., Gubelicko, C. B., Shapiro, L. H., Look, A. T., and Holmes, K. V. (1992) *Nature* **357**, 420–422
32. Delmas, B., Gelfi, J., L’Haridon, R., Vogel, L. K., Sjostrand, H., Noren, O., and Laude, H. (1992) *Nature* **357**, 417–420
33. Delmas, B., Gelfi, J., Sjostrand, H., Noren, O., and Laude, H. (1993) *Adv. Exp. Med. Biol.* **319**, 295–305
34. Nomura, R., Kiyota, A., Suzaki, E., Kataoka, K., Ohe, Y., Miyamoto, K., Sendai, T., and Fujimoto, T. (2004) *J. Virol.* **78**, 8701–8708
35. Inoue, J., Goto, S., Nomura, S., Isobe, K., Nawa, A., Okamoto, T., and Tomoda, Y. (1995) *Anticancer Res.* **15**, 2081–2087
36. Kowalski, J., Belowski, D., Madej, A., and Herman, Z. S. (1995) *Arch. Immunol. Ther. Exp.* **43**, 265–271
37. Moffatt, S., Wiehle, S., and Cristiano, R. J. (2005) *Hum. Gene Ther.* **16**, 57–67
38. Inohashi, K., Tsuchiya, T., Harada, M., and Nishio, Y. (1999) *Acta Haematol.* **97**, 94–97
39. Yamagishi, H., Naito, K., Ueda, Y., Ito, K., Kohnosu, H., Kobayashi, M., Kubo, H., Matsuda, A., and Ok, T. (1991) *Biomed. Pharmacother.* **45**, 117–120
40. Mina-Osorio, P., and Ortega, E. (2005) *J. Leukocyte Biol.* **77**, 1008–1017
41. Lendeckel, U., Arndt, M., Frank, K., Wex, T., and Ansorge, S. (1999) *Int. J. Mol. Med.* **4**, 17–27
42. Woodhead, V. E., Stonehouse, T. J., Binks, M. H., Speidel, K., Fox, D. A., Gaya, A., Hardie, D., Hemminki, A., Horeji, V., Sagawa, K., Skubitz, K. M., Taskov, H., Todd, R. F., III, van Agthoven, A., Katz, D. R., and Chain, B. M. (2000) *Int. Immunol.* **12**, 1051–1061
43. Bank, U., Tadje, J., Helmut, M., Stefan, T., Tager, M., Wolke, C., Wischeropp, A., Ittensohn, A., Reinhold, D., Ansorge, S., and Lendeckel, U. (2006) *Exp. Med. Biol.* 575, in press
44. Lendeckel, U., Wex, T., Reinhold, D., Kahne, T., Frank, K., Faust, J., Neubert, K., and Ansorge, S. (1996) *Biochem. J.* **319**, 817–821
45. Lendeckel, U., Wex, T., Arndt, M., Frank, K., Franke, A., and Ansorge, S. (1998) *Hum. Mutat.* **1**, S158–S160
46. Jacob, R., Alfaiah, M., Grunberg, J., Obendorf, M., and Naim, H. Y. (2000) *J. Biol. Chem.* **275**, 6566–6572
47. Fenteany, G., Standaert, R. F., Lane, W. S., Choi, S., Corey, E. J., and Schreiber, S. L. (1995) *Science* **268**, 726–731
48. Jacob, R., Brewer, C., Fransen, J. A., and Naim, H. Y. (1994) *J. Biol. Chem.* **269**, 2712–2721
49. Firla, B., Arndt, M., Frank, K., Thiel, U., Ansorge, S., Tager, M., and Lendeckel, U. (2002) *Free Radic. Biol. Med.* **32**, 584–595
50. Krueger, S., Hundertmark, T., Kalinski, T., Peitz, U., Wex, T., Naumann, M., and Roesner, A. (2005) *J. Biol. Chem.* **281**, 2868–2875
51. Mina-Osorio, P., and Ortega, E. (2005) *Cell* **12**, 1051–1061