# A Phosphatase Associated with Metastasis of Colorectal Cancer

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5′-triphosphate (ATP), 100 mM KCl, and 1 mM dithiothreitol (DTT). Samples were incubated at 37°C for 5 min, fixed with 0.5% glutaraldehyde, and squashed under cover slips.
21. An Olympus VE-DIC equipped with Plan Apo 60×/1.4 numerical aperture DIC lens and Hamamatsu 2400 newvicon video camera was used. Images were enhanced with an Argus 20 (Hamamatsu). Centrosomes were first perfused into perfusion chamber on ice. Perfusion chamber surfaces were blocked by incubation with 5 mg/ml casein in BRB80 and washed with 0.5% NP-40 in BRB80. Microtubule assembly was initiated by perfusion of 33 μM tubulin in BRB80 containing 1 mM GTP, 1.5 mM ATP, 100 mM KCl, 1 mM DTT, and 0.5 mg/ml casein. Subsequently, tubulin with purified proteins or control buffer was perfused, and the fate of microtubules was monitored.
22. Modified from the perfusion assay described in (*21*). After absorption of centrosomes, blocking with casein, and washing with NP-40, 25 μM tubulin with purified proteins or control buffer in BRB80 containing 1 mM GTP, 1.5 mM ATP, 60 mM KCl, 1 mM DTT, 0.5% NP-40, and 0.5 mg/ml casein were perfused into the chamber on ice. The perfusion chamber was warmed to 30°C and sealed. Recording was performed at 30°C every 1 s for 10 min. Analysis of microtubule dynamics was done as described (*10*). Catastrophe frequency was calculated by dividing the total number of events by the duration of growth and pause phases.
23. D. L. Gard, M. W. Kirschner, *J. Cell Biol.* **105**, 2191 (1987).
24. K. Nabeshima *et al.*, *Genes Dev.* **9**, 1572 (1995).
25. P. J. Wang, T. C. Huffaker, *J. Cell Biol.* **139**, 1271 (1997).
26. S. Charrasse *et al.*, *J. Cell Sci.* **111**, 1371 (1998).
27. L. R. Matthews, P. Carter, M. D. Thierry, K. Kemphues, *J. Cell Biol.* **141**, 1159 (1998).
28. C. F. Cullen, P. Deak, D. M. Glover, H. Ohkura, *J. Cell Biol.* **146**, 1005 (1999).
29. R. Graf, C. Daunderer, M. Schliwa, *J. Cell Sci.* **113**, 1747 (2000).
30. L. Wordeman, T. J. Mitchison, *J. Cell Biol.* **128**, 95 (1995).
31. F. Severin, B. Habermann, T. Huffaker, T. Hyman, *J. Cell Biol.* **153**, 435 (2001).
32. We are grateful to C. E. Walczak for the cDNA clone of XKCM1; S. Berthold and F. Senger for help with protein purification; E. Karsenti and his group for helpful discussion; and H. Funabiki, J. Howard, K. Oegema, E. Tanaka, and W. Zachariae for comments on the manuscript. Supported by a Human Frontier Science Program Organization long-term fellowship and a grant from the Deutsche Forschungsgemeinschaft (K.K.), a Max-Planck-Gesellschaft fellowship and a grant from the Fondation pour la Recherche Médicale (I.A.), and fellowships from the European Molecular Biology Organization and the American Cancer Society (A.D.).

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# A Phosphatase Associated with Metastasis of Colorectal Cancer

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**To gain insights into the molecular basis for metastasis, we compared the global gene expression profile of metastatic colorectal cancer with that of primary cancers, benign colorectal tumors, and normal colorectal epithelium. Among the genes identified, the *PRL-3* protein tyrosine phosphatase gene was of particular interest. It was expressed at high levels in each of 18 cancer metastases studied but at lower levels in nonmetastatic tumors and normal colorectal epithelium. In 3 of 12 metastases examined, multiple copies of the *PRL-3* gene were found within a small amplicon located at chromosome 8q24.3. These data suggest that the *PRL-3* gene is important for colorectal cancer metastasis and provide a new therapeutic target for these intractable lesions.**

Metastasis is the neoplastic process responsible for most deaths from cancer because the primary tumors can usually be surgically removed. Metastatic cells undergo cytoskeletal changes, loss of adhesion, and enhanced motility and express proteolytic enzymes that degrade the basement membrane (*1*–*3*). However, much remains to be learned about this lethal process, and further progress is contingent upon identifying novel genes and pathways that are consistently and specifically altered in metastatic lesions.

In the case of colorectal tumorigenesis, the genes associated with initiation and progression to the invasive (cancerous) stage are well known (*4*). However, no gene has been shown to be consistently and specifically activated in liver metastases, the lesions that are usually responsible for the deaths of colorectal cancer patients. To learn which genes might be involved in this process, we performed global gene expression profiles of liver metastases using serial analysis of gene expression (SAGE) technology (*5*). We first prepared a SAGE library from microdissected metastases (*6*). Surprisingly, we found that many of the transcripts identified in these libraries were characteristic of normal hepatic or inflammatory cells, precluding quantitative analysis (*7*). To produce a more specific profile of metastatic epithelial cells, we developed an immunoaffinity fractionation procedure to purify colorectal epithelial cells from contaminating stromal and hepatic cells (*8*). A SAGE library was prepared from cells purified in this manner, yielding ~95,000 tags representing at least 17,324 transcripts (*6*). These tags were compared with ~4 million tags derived from diverse SAGE libraries, particularly those from normal and malignant (but nonmetastatic) colorectal epithelium (*9*). One hundred and forty-four transcripts were represented at significantly higher levels in the metastasis library than in the other libraries, while 79 transcripts were

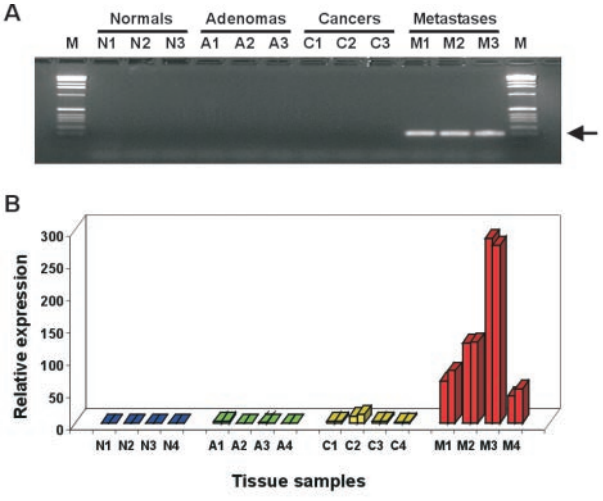


**Fig. 1.** *PRL-3* expression in human colorectal tumors of different stage. The expression of *PRL-3* was evaluated by real-time PCR (*8*) and compared with that of the β-amyloid precursor protein (*APP*) gene, shown previously to be expressed at nearly identical levels in normal and neoplastic colorectal tissues (*9*). The metastases analyzed in this experiment were derived from patients other than the ones from whom the normal epithelium and other lesions were derived. Epithelial cells were purified as described (*8*). (**A**) Gel of RT-PCR products from normal colorectal epithelium (N1 to N3), adenomas (A1 to A3), primary cancers (C1 to C3), and metastases (M1 to M3). Real-time PCR was performed for 24 cycles, when RT-PCR products from the metastases were evident but before signals from the other lesions had appeared. Arrow indicates the *PRL-3* RT-PCR product of 198 bp. Lane M, molecular size markers. (**B**) Results are expressed as the ratio between *PRL-3* and *APP* expression and are normalized to the average expression in adenomas. Duplicates are shown for each analysis.

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represented at significantly lower levels in the metastasis library (*10*).

Transcripts that were enriched rather than depleted in the metastasis library have obvious diagnostic and therapeutic potential. We therefore selected 38 of the most interesting enriched transcripts for further analysis (Table 1) (*11*). To confirm the SAGE data, we compared the expression of these transcripts in several microdissected metastases, primary cancers, pre-malignant adenomas, and normal epithelium by quantitative, real-time polymerase chain reaction (PCR) (*8*). Although all 38 of these transcripts were found to be elevated in at least a subset of the metastatic lesions tested, only one gene, *PRL-3*, was found to be consistently overexpressed.

*PRL-3* (also known as *PTP4A3*) encodes a small, 22-kD tyrosine phosphatase that is located at the cytoplasmic membrane when prenylated at its COOH-terminus and in the nucleus when it is not conjugated to this lipid (*12*). Among normal human adult tissues, it is expressed predominantly in muscle and heart (*13*). Although *PRL-3* had not been linked previously to human cancer, overexpression of *PRL-3* has been found to enhance growth of human embryonic kidney fibroblasts (*13*), and overexpression of *PRL-1* or *PRL-2*, close relatives of *PRL-3*, has been found to transform mouse fibroblasts and hamster pancreatic epithelial cells in culture and promote tumor growth in nude mice (*14*, *15*). On the basis of this information, our preliminary expression data, and the importance of other phosphatases in cell signaling and neoplasia, we examined *PRL-3* in greater detail. We first investigated *PRL-3* expression in epithelial cells purified from colorectal tissues from various stages of colorectal neoplasia using the procedure described above (*8*); this purification proved essential for accurate quantification of *PRL-3* expression. *PRL-3* was expressed at low levels in normal colorectal epithelium and epithelium from benign tumors (adenomas) and at intermediate levels in a subset of malignant stage I or II cancers (Fig. 1). In contrast, it was expressed at relatively high levels in each of 12 colorectal cancer metastases (examples in Fig. 1).

The metastases evaluated in Fig. 1 were not derived from the same patients from whom the earlier stage lesions were obtained. Previous analyses have revealed that expression patterns of many genes differ between individuals, and that comparisons of tissues from different patients could potentially be misleading (*16*). To address this concern, we purified epithelial cells from a liver metasta-

**Table 1.** Selected genes up-regulated in colon cancer metastasis. SAGE TAG refers to the 10–base pair (bp) sequence immediately adjacent to the Nla III restriction site (CATG) in each transcript. SAGE libraries were constructed from two normal colonic tissues (one library containing 48,479 tags, the other 49,610 tags), two primary colorectal cancers (one library containing 55,700 tags, the other 41,371 tags), and a colorectal metastasis (94,445 tags). Transcript description refers to the Unigene assignment of the SAGE TAG (www.ncbi.nlm.nih.gov/SAGE/SAGEtag.cgi). When tags matched two Unigene assignments, both are listed. The numbers listed in each column refer to the number of SAGE tags corresponding to the indicated gene that were observed in the library made from the indicated tissue. For example, 12 SAGE tags were observed for *PRL-3* in the SAGE library from the metastasis, but no *PRL-3* SAGE tags were observed in the other four libraries. EST, expressed sequence tag.

| SAGE Tag | Normal 1 | Normal 2 | Cancer 1 | Cancer 2 | Metastasis | Description |
|---|---|---|---|---|---|---|
| TAGGTCAGGA | 0 | 0 | 0 | 0 | 12 | Protein tyrosine phosphatase type IVA (PRL-3) |
| TGATTGGTTT | 0 | 0 | 0 | 0 | 11 | cDNA: FLJ23603 |
| GGAATATGCA | 0 | 0 | 0 | 1 | 18 | LOC54675: hypothetical protein |
| AATCTTGTTT | 0 | 0 | 0 | 0 | 9 | Hypothetical gene ZD52F10 |
| GTCTTCTTAA | 0 | 0 | 1 | 0 | 16 | DNAJ domain–containing |
| ATAATAAAAG | 0 | 0 | 1 | 1 | 30 | GRO3 oncogene/T45117 hU1-70K protein |
| TAAATATGGA | 0 | 0 | 0 | 0 | 7 | Attractin |
| ATTTTTGTAT | 0 | 0 | 1 | 0 | 14 | Bcl-2 binding component 3 |
| TAGCTGGAAA | 4 | 0 | 0 | 1 | 67 | Nuclear receptor subfamily 4 |
| GGAGGGCTGG | 0 | 0 | 1 | 0 | 13 | Mitogen-activated protein kinase 8 interacting protein 2/ESTs similar to S26689 |
| GTCAGTCACT | 1 | 0 | 0 | 0 | 13 | Hairy (*Drosophila*) homolog |
| TTGAGTAGGA | 1 | 0 | 1 | 0 | 23 | LUC7 (*Saccharomyces cerevisiae*)-like |
| TGGGGGCCGA | 0 | 3 | 0 | 0 | 33 | Transducin-like enhancer of split 2, homolog of *Drosophila* E (sp1) |
| TGGGCTGGGG | 0 | 0 | 2 | 1 | 32 | Adipose differentiation-related protein |
| TAGCTGGAAC | 0 | 0 | 1 | 0 | 10 | ESTs, no known homologies |
| CTTCCTTGCC | 0 | 0 | 2 | 3 | 46 | Keratin 17 |
| GCGGCAGTTA | 0 | 0 | 0 | 1 | 8 | Casein kinase 2, alpha prime polypeptide |
| CTGCACTTAC | 1 | 0 | 2 | 3 | 46 | Minichromosome maintenance deficient 7 (*S. cerevisiae*) |
| AAGCTGTTTA | 1 | 0 | 1 | 1 | 23 | v-Jun avian sarcoma virus 17 oncogene homolog/LSFR2 gene 2/MGC2550 protein |
| GTGAGGGCTA | 1 | 2 | 1 | 0 | 28 | Plexin B1 |
| GGGGCTGTAT | 0 | 0 | 1 | 0 | 7 | Transforming growth factor, beta 1 (TGF-β) |
| CTGGAGGCTG | 1 | 1 | 0 | 1 | 20 | ESTs, similar to GTP-rho binding protein 1 (rhophilin) |
| GGCTGGGTTT | 1 | 1 | 1 | 5 | 42 | H2.0 (*Drosophila*)-like homeobox 1 |
| GGGGGTGGGT | 1 | 0 | 0 | 1 | 10 | Mago-nashi (*Drosophila*) homolog, proliferation-associated |
| CAGCATCTAA | 1 | 1 | 1 | 1 | 19 | Putative Rab5-interacting protein |
| TTTCCAATCT | 0 | 0 | 0 | 4 | 18 | Vascular endothelial growth factor |
| TTTCTAGGGG | 0 | 1 | 2 | 2 | 22 | PTD008 protein |
| AAAGTGAAGA | 1 | 0 | 3 | 5 | 37 | FLJ11328 protein/ribosomal protein L10 |
| TTTGCACTTG | 3 | 1 | 0 | 0 | 15 | wee1+ (*Schizosaccharomyces pombe*) homolog/protein x 013 |
| CCTGGAATGA | 1 | 0 | 0 | 3 | 15 | cDNA: FLJ12683 |
| GGAGGTAGGG | 1 | 0 | 3 | 0 | 15 | PTK7 protein tyrosine kinase 7 |
| CTGTACTTGT | 9 | 5 | 0 | 0 | 52 | v-fos FBJ murine osteosarcoma viral oncogene homolog B |
| TCCTTGCTTC | 1 | 1 | 3 | 1 | 22 | FLJ20297 protein |
| TATCTGTCTA | 0 | 0 | 2 | 5 | 25 | SET translocation (myeloid leukemia–associated) |
| TTAGATAAGC | 0 | 0 | 3 | 8 | 33 | Chaperonin-containing TCP1, subunit 6A (zeta 1) |
| AGTGGAGGGA | 1 | 0 | 1 | 1 | 9 | Ataxin 2 related protein |
| TGCAGATATT | 0 | 0 | 2 | 1 | 8 | Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual-specificity phosphatase) |
| GGGAGGGGTG | 1 | 1 | 2 | 2 | 14 | Matrix metalloproteinase 14 (membrane-inserted) |

sis, the advanced (stage III) colorectal cancer giving rise to the metastasis, and normal colorectal epithelium from each of six different patients. There was little or no expression in the normal epithelium from these cases, intermediate expression in the advanced primary cancers, and significantly higher expression in each of the matched metastatic lesions (Fig. 2).

The observation that *PRL-3* is expressed at relatively high levels in metastases is consistent with its playing a causative role in metastasis. However, the most definitive way to implicate a gene in human cancer is to identify genetic alterations of that gene (*17*). We therefore determined whether *PRL-3* was genetically altered through gene amplification, a well-known mechanism for increasing the expression of growth-regulating genes in human cancers (*18*). Through radiation hybrid and syntenic mapping, we found that the *PRL-3* gene was located ~3 Mb from the telomere of chromosome 8q, at a position corresponding to chromosomal band 8q24.3 (*19*). Real-time PCR analyses of genomic DNA prepared from the purified epithelial cells of 12 metastatic lesions, each from a different patient, was performed to determine *PRL-3* gene content; as with expression analyses, such purification proved critical for reliable quantification. In each case, we determined the genomic content of *PRL-3* sequences relative to that of a sequence near the centromere of chromosome 8q (*8*). This comparison allowed us to distinguish true amplification from simple increases in chromosome number due to aneuploidy (*18*). We found that 3 of the 12 metastases studied exhibited amplification, to levels of ~25 copies, 26 copies, and 37 copies per diploid genome.

We next compared the genomic content of sequences distributed throughout chromosome 8q to define those that were amplified in these three metastases (*8*). The availability of the nearly complete human genomic sequence considerably facilitated this mapping effort (*20*, *21*). Although the *PRL-3* gene was not found in public databases generated through the Human Genome Project, it was found on a Celera scaffold located ~145 Mb from the telomere of chromosome 8p and ~3 Mb from the telomere of chromosome 8q, consistent with our radiation hybrid mapping data (*19*, *22*) (Fig. 3). In all cases, amplification was confined to a very small region of chromosome 8q that included *PRL-3* (Fig. 3) (*22*). The only known or predicted genes within this small amplicon were *PRL-3* and a hypothetical gene homologous to a TATA-binding protein. No expression of the latter gene was detectable in metastatic lesions when assessed by reverse transcription (RT)–PCR. However, the region of the Celera scaffold on which *PRL-3* maps (*22*) contains gaps estimated to be <20 kb in size. If these gaps were in fact larger than expected, the total size of the amplicon would be larger than the 100 kb indicated in Fig. 3 and could contain additional genes.

In summary, we have identified a gene encoding a tyrosine phosphatase that is consistently overexpressed in metastatic colorec-

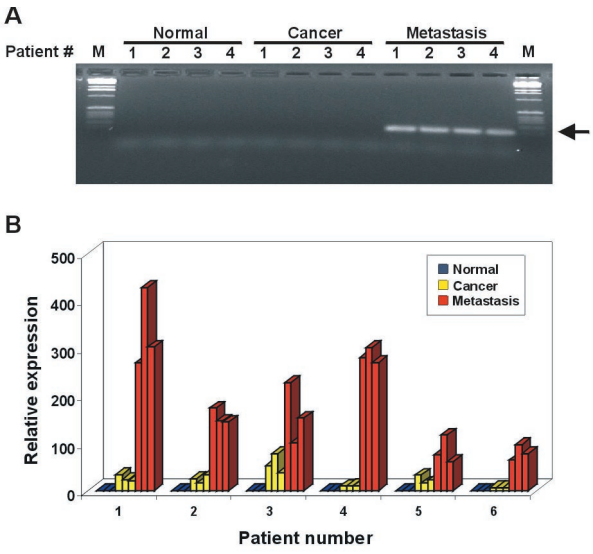


**Fig. 2.** *PRL-3* expression in matched metastases and advanced primary cancers. Colonic epithelial cells were purified from a liver metastasis, a primary cancer giving rise to the metastasis, and normal colorectal epithelium from each of six patients. (**A**) Gel of RT-PCR products from matched normal, primary cancer, and metastasis from four patients. Real-time PCR was performed for 24 cycles, when RT-PCR products from the metastases were evident but before signals from the other lesions had appeared. Arrow indicates the *PRL-3* RT-PCR product of 198 bp. Lane M, molecular size markers. (**B**) Relative levels of expression were determined as in Fig. 1.

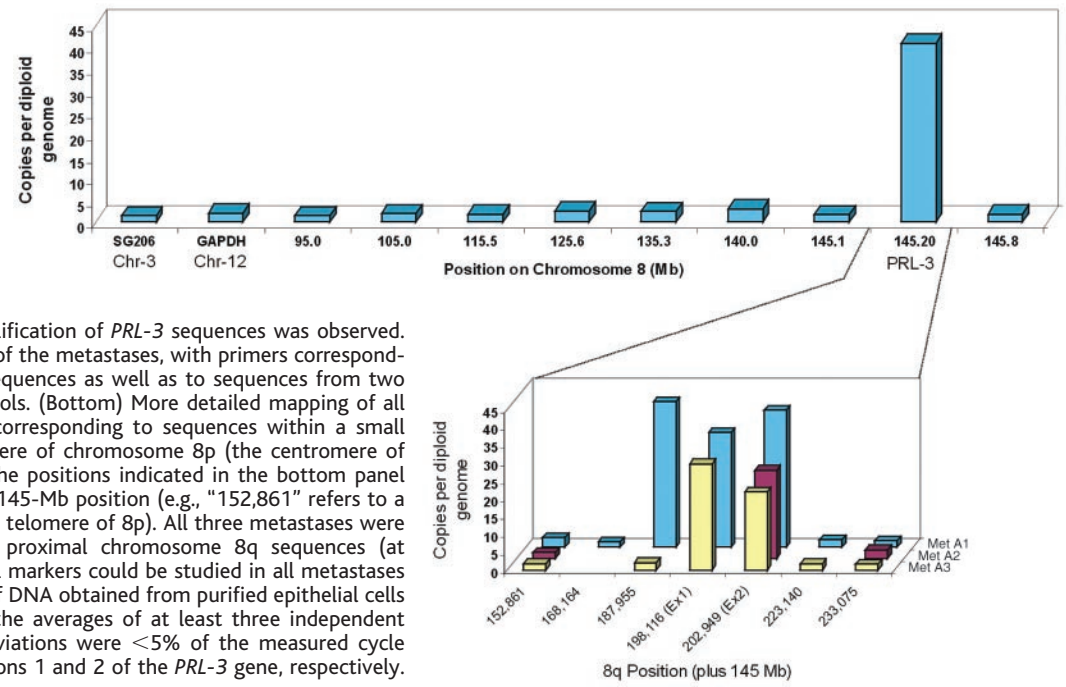


**Fig. 3.** Amplicon mapping of *PRL-3*. The number of copies of sequences corresponding to each of the indicated loci was determined by real-time PCR. In each case, the number of copies per diploid genome was determined by comparison with equal amounts of DNA from normal cells (*8*). Genomic DNA from purified epithelial cells of metastases from three different patients was used for these experiments; in another nine patients, no amplification of *PRL-3* sequences was observed. (Top) Results obtained from one of the metastases, with primers corresponding to several chromosome 8q sequences as well as to sequences from two other chromosome arms as controls. (Bottom) More detailed mapping of all three metastases, with primers corresponding to sequences within a small region ~145 Mb from the telomere of chromosome 8p (the centromere of chromosome 8 is at ~45 Mb). The positions indicated in the bottom panel refer to nucleotides distal to the 145-Mb position (e.g., "152,861" refers to a position 145,152,861 bp from the telomere of 8p). All three metastases were shown to lack amplification of proximal chromosome 8q sequences (at positions 95 and 135 Mb). Not all markers could be studied in all metastases because of the limited amounts of DNA obtained from purified epithelial cells of these lesions. Bars represent the averages of at least three independent determinations, and standard deviations were <5% of the measured cycle numbers. Ex1 and Ex2 refer to exons 1 and 2 of the *PRL-3* gene, respectively.

tal cancers. The confinement of the gene to a small amplicon in a subset of these tumors provides important, complementary evidence for the role of this gene in metastasis; the major genes previously shown to be amplified in naturally occurring cancers are all oncogenes (*18*, *23*, *24*). Extra copies of chromosome 8q DNA sequences have been observed in the advanced stages of many different tumor types, including advanced colon cancers (*25–28*). It has been suggested that the c-*MYC* gene on chromosome 8q24.12 is the target of such 8q overrepresentation. In the three metastatic lesions we examined, c-*MYC* was not amplified and was in fact located ~14 Mb from the boundaries of the *PRL-3* amplicon. It will, therefore, be of interest to evaluate the expression and genomic representation of *PRL-3* in the metastases of other cancer types.

Further experiments will be required to determine the biochemical mechanisms through which *PRL-3* influences neoplastic growth and to establish its causative role in the metastatic process. However, one of the most important ramifications of the work described here concerns its potential therapeutic implications. Most of the previously described genetic alterations in colorectal cancers involve inactivation of tumor suppressor genes. The proteins produced from these genes are difficult to target with drugs, because they are inactive or absent in the cancer cells (*29*). In contrast, enzymes whose expression is elevated in cancer cells, like that encoded by *PRL-3*, provide excellent targets for drug discovery purposes.

**References and Notes**


1. L. Weiss, *Cancer Metastasis Rev.* **19**, 193 (2000).
2. I. J. Fidler, *Surg. Oncol. Clin. N. Am.* **10**, 257, vii (2001).
3. A. Ridley, *Nature* **406**, 466 (2000).
4. K. W. Kinzler, B. Vogelstein, *Cell* **87**, 159 (1996).
5. V. E. Velculescu, B. Vogelstein, K. W. Kinzler, *Trends Genet.* **16**, 423 (2000).
6. SAGE libraries were generated with MicroSAGE, as described in a protocol available at www.sagenet.org/sage_protocol.htm. Reagents for this procedure were obtained from the I-SAGE kit available from Invitrogen (Carlsbad, California).
7. Examples of the nonepithelial transcripts identified in the initial libraries are vitronectin and lysozyme. These genes are transcribed in stromal cells and accounted for 0.4 and 0.1%, respectively, of the tags in SAGE libraries prepared from unpurified metastatic lesions but were not found in the SAGE library derived from purified metastatic epithelial cells. Similarly, transcripts made in hepatocytes, like apolipoprotein C–III, accounted for 0.3% of the tags from unpurified metastasis libraries but were not found in the libraries from purified cells. These differences in vitronectin, lysozyme, and apoliprotein C–III expression among SAGE libraries derived from purified and unpurified epithelial cells were statistically significant ($P < 0.0001$, $\chi^2$ test).
8. Methods used in this study, including those used for purification of epithelial cells, are described in the supplementary Web material available on *Science* Online at www.sciencemag.org/cgi/content/full/1065817/DC1.
9. V. E. Velculescu *et al.*, *Nature Genet.* **23**, 387 (1999).
10. The metastasis-derived library was compared with two primary colorectal cancer and two normal colorectal epithelial libraries (*30*). Monte Carlo simulations were used to identify transcripts that were expressed in the metastasis library at levels 10-fold higher or 10-fold lower than in the other libraries, with *P*-chance <0.0001, as described (*31*). A complete listing of the SAGE tags and corresponding transcripts identified in this study is available at www.sagenet.org.
11. The transcripts chosen for further analysis included those for which the SAGE data indicated at least 10-fold greater expression in metastatic versus nonmetastatic lesions or for which the predicted gene products had potentially interesting functional properties.
12. Q. Zeng *et al.*, *J. Biol. Chem.* **275**, 21444 (2000).
13. W. F. Matter *et al.*, *Biochem. Biophys. Res. Commun.* **283**, 1061 (2001).
14. R. H. Diamond, D. E. Cressman, T. M. Laz, C. S. Abrams, R. Taub, *Mol. Cell. Biol.* **14**, 3752 (1994).
15. C. A. Cates *et al.*, *Cancer Lett.* **110**, 49 (1996).
16. C. M. Perou *et al.*, *Nature* **406**, 747 (2000).
17. D. Haber, E. Harlow, *Nature Genet.* **16**, 320 (1997).
18. G. M. Brodeur, M. D. Hogarty, in *The Genetic Basis of Human Cancer*, K. W. Kinzler, B. Vogelstein, Eds. (McGraw-Hill, New York, 1998), vol. 1, pp. 161–179.
19. The mouse homolog of *PRL-3* was mapped to a region of mouse chromosome 15 [23135.11cR on chromosome 15 in the radiation hybrid map of the Whitehead Institute Map (cR3000)] that is syntenic to the human 8q22 -8q24.3 (www.ncbi.nlm.nih.gov/Homology/view.cgi?chr=15&tax_id=10090). STS (sequence tagged site) primers from the *PRL-3* gene and surrounding marker 145.8 (Fig. 3) were mapped to the Stanford G3 radiation hybrid panel. Both were shown to be tightly linked to marker SHGC-22154, located at 8q24.3, ~3 Mb from the 8q telomere. In comparison, c-*Myc* was mapped to 8q24.12-q24.13 in the Stanford G3 map, ~17 Mb from the telomere.
20. J. C. Venter *et al.*, *Science* **291**, 1304 (2001).
21. E. S. Lander *et al.*, *Nature* **409**, 860 (2001).
22. The *PRL-3* transcript (GenBank accession number NM_032611) could not be identified in the Human Genome Project draft genome sequences, using standard tools including BLAST searches against the draft genome sequence (www.ncbi.nlm.nih.gov/genome/seq/page.cgi?F=HsBlast.html&&ORG=Hs) and BLAT analyses of the assembled draft sequences (http://genome.ucsc.edu/cgi-bin/hgBlat?command=start). However, the *PRL-3* transcript was identical to Celera transcript hCT11716 and was found on Celera scaffold GA_x2KMHMRCHQ1, CHGD Assembly Release 25h (www.celera.com).
23. M. A. Heiskanen *et al.*, *Cancer Res.* **60**, 799 (2000).
24. M. D. Pegram, G. Konecny, D. J. Slamon, *Cancer Treat. Res.* **103**, 57 (2000).
25. J. C. Alers *et al.*, *Lab Invest.* **77**, 437 (1997).
26. A. Paredes-Zaglul *et al.*, *Clin. Cancer Res.* **4**, 879 (1998).
27. N. N. Nupponen, J. Isola, T. Visakorpi, *Genes Chromosomes Cancer* **28**, 203 (2000).
28. A. El Gedaily *et al.*, *Prostate* **46**, 184 (2001).
29. B. Vogelstein, D. Lane, A. J. Levine, *Nature* **408**, 307 (2000).
30. L. Zhang *et al.*, *Science* **276**, 1268 (1997).
31. V. E. Velculescu, L. Zhang, B. Vogelstein, K. W. Kinzler, *Science* **270**, 484 (1995).


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# Kinetic Stabilization of the α-Synuclein Protofibril by a Dopamine–α-Synuclein Adduct


**Kelly A. Conway,* Jean-Christophe Rochet,* Robert M. Bieganski, Peter T. Lansbury Jr.†**



**The substantia nigra in Parkinson's disease (PD) is depleted of dopaminergic neurons and contains fibrillar Lewy bodies comprising primarily α-synuclein. We screened a library to identify drug-like molecules to probe the relation between neurodegeneration and α-synuclein fibrilization. All but one of 15 fibril inhibitors were catecholamines related to dopamine. The inhibitory activity of dopamine depended on its oxidative ligation to α-synuclein and was selective for the protofibril-to-fibril conversion, causing accumulation of the α-synuclein protofibril. Adduct formation provides an explanation for the dopaminergic selectivity of α-synuclein–associated neurotoxicity in PD and has implications for current and future PD therapeutic and diagnostic strategies.**


A central role for α-synuclein fibrilization in PD (*1, 2*) is supported by the fact that fibrillar α-synuclein is a major component of Lewy bodies (*3, 4*) and that the α-synuclein gene has been linked to autosomal dominant PD (FPD) (*5, 6*). Although both FPD α-synuclein mutations [$Ala^{53} \rightarrow$ Thr (A53T) and $Ala^{30} \rightarrow$ Pro (A30P)] accelerate the formation of nonfibrillar, oligomeric protofibrils in vitro, A30P inhibits the conversion of protofibrils to fibrils (*7*). This finding suggests that protofibrils may be pathogenic and fibrils inert, or even protective (*2*). Transgenic mice that express wild-type human α-synuclein (*8*) exhibit PD-like dopaminergic deficits and motor dysfunction, and they develop neuronal inclusions that, in contrast to those