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Atherosclerosis, just another cancer?

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Editorial

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Atherosclerosis and cancer are the two leading causes of death in the industrialized world. A most unexpected manuscript in this issue of *The Journal of Clinical Investigation* makes the observation that inactivation of one growth regulatory gene, the type II TGF-β receptor (RII), may be a central lesion in the pathobiology of both diseases (1). Indeed, both may be traced to an identical mutation in the RII gene.

The TGF-βs define a large superfamily of secreted ligands that play key roles in development and in growth regulation (2–5). In humans, these ligands include the TGF-βs proper, the activins, the inhibins, and the bone morphogenic proteins. All of these hormones share in common both a conserved spacing of seven cysteine residues and a dimeric structure that comprises the physiologically active mature ligands. The direct TGF-β family (referred to collectively as TGF-β) is composed of TGF-β1, 2, and 3, which are encoded from three separate genes (2–5). The TGF-βs are well known as potent inhibitors of cell growth, characteristically inducing cell growth arrest in the late G1 phase of the cell cycle (2–4, 6). TGF-βs are particularly potent inhibitors of epithelial cell growth, both in vitro and in vivo (2–4, 7, 8). Indeed, in some epithelial cell types TGF-β can induce a cascade of apoptotic cell death (9). TGF-β also acts to suppress the immune system, to promote wound healing, and, in some studies, to suppress atherogenesis (2–4).

At least a few of the biological activities of TGF-β are mediated via induction of gene transcription (2–5). For example, TGF-β growth inhibition in different cell types is accompanied by induction of transcription of the cyclin kinase inhibitors p21 and p15 (10, 11). TGF-β also prominently induces expression of a variety of genes involved in cell matrix deposition and attachment, including collagen, integrins, and plasminogen activator inhibitor-1 (2–4).

The TGF-β superfamily of ligands shares the trait of signaling through a related superfamily of cell surface receptors that are all serine-threonine kinases. TGF-β1, 2, and 3 all bind specifically to a single common cell surface receptor complex composed of type I (RI) and type II (RII) subunits that are encoded from separate genes. TGF-β binds first to the RII subunit, which in turn assembles with and phosphorylates serine and threonine residues on the RI subunit (12). The ensuing activation of the RI kinase is an obligate requirement for further cellular transduction of the TGF-β–mediated signal (12).

Direct evidence for the role of the TGF-β growth suppressor pathway as a tumor suppressor pathway resulted from the finding of RII mutations in human colon cancer (13, 14). In these tumors, somatic mutations were acquired in RII during the process of carcinogenesis, resulting in inactivation of both RII alleles (14). Characteristically, these were frameshift mutations that encoded for a truncated RII protein lacking a kinase domain and, therefore, defective in signaling (14, 15). In colon cancer cell lines, restoration by gene transfection of wild-type RII signaling abolished the ability of cells to form tumors in athymic mice (16). Thus, RII behaves as a colon cancer tumor suppressor gene by both genetic and functional criteria.

RII mutations seen among colon cancers were almost all frameshift mutations clustered within a 10-bp polyadenine run that encodes a three codon lysine repeat (14). Moreover, cancers with these mutations all proved to be tumors that also demonstrated a form of genomic instability known as microsatellite instability (or replication errors [RER]; 14). In such tumors, defects in a DNA repair system (more properly, the DNA mismatch repair system), result in a marked susceptibility to frameshift mutations of repetitive DNA sequences, particularly within long noncoding DNA repeats known as microsatellites (17–21). RII mutations were found in $> 90\%$ of such RER colon cancers (14, 15).

Significantly, in rare tumors one of the RII alleles was found to be inactivated by mutation outside to the RII polyadenine repeat, demonstrating that inactivation of the gene is selected for, and therefore is not simply an epiphenomenon of the RER phenotype (15). This point has been confirmed by subsequent reports of other RII point mutations outside of the polyadenine repeat that have inactivated the RII gene in lymphomas (22) and in head and neck tumors (23). Thus, the general paradigm that emerges is that mutation of RII abrogates TGF-β negative regulation of cell growth, leading to cell proliferation and to neoplasia.

The findings by McCaffrey and colleagues (1) now extend this very same model to the process of atherogenesis and to restenosis of vessels after angioplasty. Specifically, these investigators report detecting frameshift mutations in the RII coding region polyadenine repeat in some samples of human atherosclerotic lesions and of human restenotic lesions that were acquired from both carotid and coronary arteries. Moreover, the authors also detect in one case an RII point mutation that alters the charge of the encoded residue.

These observations have multiple implications regarding the pathophysiology of atherogenesis. First, the detection of any gene mutation in these tissues establishes that in both atheromas and in restenotic vascular lesions a substantial percentage of the cells must be derived from an original mutant progenitor cell, and therefore comprise a monoclonal population. As such, both atheromas and restenotic lesions could be properly regarded as neoplasms.

Second, the finding of RII mutations in vascular lesions strongly suggests that, just as it functions as a tumor suppressor in the gut, TGF-β also functions as an atherogenesis suppressor in blood vessels. This model is functionally consistent with previous studies in animal models in which increased TGF-β levels appeared to be protective against atherogenesis (24). Such a finding has obvious implications both for development of drugs or gene-based therapeutics that might protect vessels against atherogenesis or restenosis. Interestingly, a significant role for the TGF-β superfamily in the regulation of blood vessel formation has been suggested from the finding that individuals with hereditary hemorrhagic telangiectasia carry germ

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line mutations either in Alk-1, a member of the superfamily of receptors related to the TGF-β receptor, or in endoglin, an endothelial cell protein important in initial cellular binding of TGF-β (25, 26).

Finally, the finding of mutations within the TGF-β coding region polyadenine repeat has interesting implications for the mechanism of RII mutation in atherogenesis. It will certainly be of interest to elucidate whether this observation is only the tip of the iceberg, and will be followed by finding in vascular lesions that mutations are common throughout RII, within RI, or in downstream components of TGF-β signaling, or whether these observations reflect a unique susceptibility of the RII polyadenine repeat to mutagenesis. If the latter, it will be important to determine whether there is a key environmental mutagen that induces these lesions, and hence initiates atherogenesis. The data of McCaffrey and colleagues (1) suggest that these vascular lesions do not betray the widespread instability of repetitive DNA sequences that is typical of tumors that arise via inactivation of DNA mismatch repair. Moreover, as mutation in the RII polyadenine tract has not been seen previously in any tumor cells in which DNA repair is proficient, it is most tempting to speculate on the possibility that an environmental cofactor contributes to those mutations that arise in blood vessels. Previous studies of p53 mutations in cancers support the idea that repetitive DNA tracts can be hot spots for de novo mutation in repair proficient cells, but have also left unanswered whether that is due to a propensity for polymerase errors within such sequences or to the activity of specific classes of mutagens (27).

Given the potential implications of the findings of McCaffrey and colleagues (1), it is reasonable to consider those points at which confirmation of these exciting findings will be eagerly awaited. One concern known to all who work in this field is that repetitive DNA sequences are extraordinarily prone to errors arising during polymerase chain reaction (PCR) amplification, and that the likelihood of such errors is highest when the amounts of starting DNA are lowest. The authors of this report have been most cognizant of this problem, and have skillfully optimized their PCR-based assay to minimize the chance of being mislead by such error. Indeed, their verification that the mutations are present both in tissue samples and in cells cultured from the samples is most reassuring in this regard. However, the minimal amounts of DNA available from most of the samples available for study appears to have precluded the extensive repetitive assay of independent aliquots from a given sample that would provide the greatest protection against being mislead. Given the importance of these authors' observations, it is likely that attempts to repeat these studies will be soon initiated in other laboratories, and that, with luck, a number of vascular lesions large enough to provide sufficient amounts of DNA will be identified to put to rest any such concerns. Such samples will also be invaluable for studies of potential mutation in regions of RII outside the polyadenine tract.

Unfortunately, it remains unclear whether the nucleotide 790 mutation observed in this study is or is not involved in inactivating receptor function. Additionally, it will be of interest to determine in future samples whether any hint is seen of widespread microsatellite instability suggestive of an underlying DNA repair defect. The non-RII derived repetitive sequences examined by McCaffrey and colleagues (1) are eight bases in length and thus likely to be less sensitive for detection of the RER defect than are longer microsatellites such as BAT-26 or BAT-40 (15).

One thing is certain: The findings of McCaffrey and colleagues will not go unnoticed. The previous findings of RII mutations in colon cancers provided a new link between defects in pathways mediating DNA repair and mutations in pathways that directly promote tumorigenesis.

The new finding that RII mutations occur in vascular lesions as well as in cancers now links the pathophysiology of two previously most dissimilar diseases. We can deeply hope that friendly competition between investigators in these two disparate disease camps will only speed the progress toward new therapies that may ameliorate both of these most common human afflictions.

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References

1. McCaffrey, T.A., B. Du, S. Consigli, P. Szabo, P.J. Bray, L. Hartner, B.B. Weksler, T.A. Sanborn, G. Bergman, and H.L. Bush, Jr. 1997. Genomic instability in the type II TGF-β1 receptor gene in atherosclerotic and restenotic vascular cells. *J. Clin. Invest.* 100:2182–2188.

2. Roberts, A., and M. Sporn. 1990. The transforming growth factor-βs. *In* Peptide Growth Factors and Their Receptors. Handbook of Experimental Pharmacology. M. Sporn and A. Roberts, editors. Springer-Verlag, Heidelberg. 419–472.

3. Massagué, J. 1990. The transforming growth factor-beta family. *Annu. Rev. Cell Biol.* 6:597–641.

4. Moses, H., E. Yang, and J. Pietenpol. 1990. TGF-β stimulation and inhibition of cell proliferation: new mechanistic insights. *Cell.* 63:245–247.

5. Massagué, J., L. Attisano, and J. Wrana. 1994. The TGF-β family and its composite receptors. *Trends Cell Biol.* 4:172–178.

6. Alexandrow, M., and H. Moses. 1995. Transforming growth factor β and cell cycle regulation. *Cancer Res.* 55:1452–1457.

7. Sellheyer, K., J.R. Bickenbach, J.A. Rothnagel, D. Bundman, M.A. Longley, T. Krieg, N.S. Roche, A.B. Roberts, and D.R. Roop. 1993. Inhibition of skin development by overexpression of transforming growth factor β1 in the epidermis of transgenic mice. *Proc. Natl. Acad. Sci. USA.* 90:5237–5241.

8. Pierce, D., A. Gorska, A. Chtil, K. Meise, D. Page, R. Coffey, and H. Moses. 1995. Mammary tumor suppression by transforming growth factor β1 transgene expression. *Proc. Natl. Acad. Sci. USA.* 92:4254–4258.

9. Wang, C.Y., J.R. Eshleman, J.K.V. Willson, and S. Markowitz. 1995. Both TGF-β and substrate release are inducers of apoptosis in a human colon adenoma cell line. *Cancer Res.* 55:5101–5105.

10. Datto, M., Y. Li, J. Panus, D. Howe, Y. Xiong, and X.-F. Wang. 1995. Transforming growth factor β induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc. Natl. Acad. Sci. USA.* 92: 5545–5549.

11. Hannon, G., and D. Beach. 1994. $p15^{INK4B}$ is a potential effector of TGF-β-induced cell cycle arrest. *Nature (Lond.).* 371:257–261.

12. Attisano, L., and J. Wrana. 1996. Signal transduction by members of the transforming growth factor-β superfamily. *Cytokine Growth Factor Rev.* 7:327–339.

13. Markowitz, S., and A. Roberts. 1996. Tumor suppressor activity of the TGF-β pathway in human cancers. *Cytokine Growth Factor Rev.* 7:93–102.

14. Markowitz, S., J. Wang, L. Myeroff, R. Parsons, L. Sun, J. Lutterbaugh, R. Fan, E. Zborowska, K. Kinzler, B. Vogelstein, et al. 1995. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. *Science (Wash. DC).* 268:1336–1338.

15. Parsons, R., L. Myeroff, B. Liu, J.K.V. Willson, S. Markowitz, K. Kinzler, and B. Vogelstein. 1995. Microsatellite instability and mutations of the TGF-β type II receptor gene in colorectal cancer. *Cancer Res.* 55:5548–5550.

16. Wang, J., L. Sun, L. Myeroff, X. Wang, L.E. Gentry, J. Yang, J. Liang, E. Zborowska, S. Markowitz, J.K.V. Willson, and M. Brattain. 1995. Demonstration that mutation of the type II TGF-β receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cells. *J. Biol. Chem.* 270:22044–22049.

17. Eshleman, J., and S. Markowitz. 1995. Microsatellite instability in inherited and sporadic neoplasms. *Curr. Opin. Oncol.* 7:83–89.

18. Kinzler, K., and B. Vogelstein. 1996. Lessons from hereditary colorectal cancer. *Cell.* 87:159–170.

19. Kolodner, R. 1996. Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev.* 10:1433–1442.

20. Marra, G., and C.R. Boland. 1995. Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspective. *J. Natl. Cancer Inst.* 87:1114–1125.

21. Modrich, P., and R. Lahue. 1996. Mismatch repair in replication fidelity, genetic recombination and cancer biology. *Annu. Rev. Biochem.* 65:101–133.

22. Knaus, P., D. Lindemann, J. DeCoteau, R. Perlman, H. Yankelev, M. Hille, M. Kadin, and H. Lodish. 1996. A dominant inhibitory mutant of the type II transforming growth factor β receptor in the malignant progression of a cutaneous T-cell lymphoma. *Mol. Cell. Biol.* 16:3480–3489.

23. Garrigue-Antar, L., T. Muñoz-Antonia, S. Antonia, J. Gesmonde, V. Vellucci, and M. Reiss. 1995. Missense mutations of the transforming growth factor β type II receptor in human head and neck squamous carcinoma cells. *Cancer Res.* 55:3982–3987.

24. Grainger, D., C. Witchell, and J. Metcalfe. 1995. Tamoxifen elevates transforming growth factor-β and suppresses diet induced formation of lipid lesions in mouse aorta. *Nat. Med.* 1:1067–1073.

25. Johnson, D.W., J.N. Berg, M. Baldwin, C. Gallione, I. Marondel, S.-J. Yoon, T. Stenzel, M. Speer, M. Pericak-Vance, A. Diamond, et al. 1996. Mutations in the activin receptor-like kinase 1 gene in hereditary hemorrhagic telangiectasia type 2. *Nat. Med.* 13:189–195.

26. McAllister, K., K. Grogg, D. Johnson, C. Gallione, M. Baldwin, C. Jackson, E. Helmbold, D. Markel, W. McKinnon, J. Murrell, et al. 1994. Endoglin, a TGF-β binding protein of endothelial cells, is the gene for hereditary hemorrhagic telangiectasia type 1. *Nat. Genet.* 8:345–351.

27. Greenblatt, M., A. Grollman, and C. Harris. 1996. Deletions and insertions in the p53 tumor suppressor gene in human cancers: confirmation of the polymerase slippage/misalignment model. *Cancer Res.* 56:2130–2136.