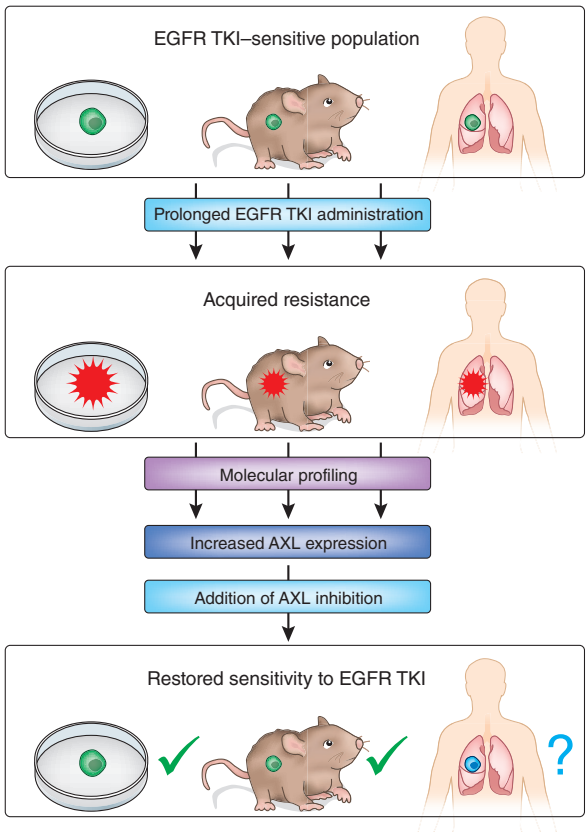

Katie Vicari

Figure 1 Acquired resistance to EGFR-targeted TKIs caused by activation of the AXL kinase. *In vitro* and *in vivo* models suggest that AXL activation is a mechanism of acquired resistance to EGFR inhibitors in NSCLC and that its inhibition can restore drug sensitivity. Determining whether targeting AXL could also overcome acquired resistance in the clinical setting is now the biggest challenge.

the development of acquired resistance, as well as by the risk of disease flare at EGFR inhibitor cessation[12]. However, whether an AXL inhibitor might replace the EGFR TKI and allow EGFR TKI holidays would need further preclinical evaluation. The possibility of 're-response' after an EGFR inhibitor–free period associated with disappearance of the mutations conferring acquired resistance[4] indicates that sequential treatment with transitory withdrawal of selective pressure from the EGFR TKI could be used.

Phenotypic changes

From a mechanistic point of view, the role of GAS6 and AXL downstream signaling pathways and the characteristics of EMT associated with AXL upregulation upon resistance remain to be established. Phenotypic changes are an important aspect of acquired drug resistance, as evidenced by a SCLC phenotype that emerges in some cases of EGFR inhibitor resistance[4]. Thus, further investigations of mechanisms that could foster an EMT feedback loop through AXL are required.

If overcoming resistance to molecularly targeted agents for the treatment of cancer is to be achieved, the precise identification of relevant resistance mechanisms is required to establish robust and durable therapies.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Lynch, T.J. *et al. N. Engl. J. Med.* **350**, 2129–2139 (2004).
2. Paez, J.G. *et al. Science* **304**, 1497–1500 (2004).
3. Pao, W. *et al. Proc. Natl. Acad. Sci. USA* **101**, 13306–13311 (2004).
4. Sequist, L.V. *et al. Sci. Transl. Med.* **3**, 75ra26 (2011).
5. Oxnard, G.R. *et al. Clin. Cancer Res.* **17**, 1616–1622 (2011).
6. Zhang, Z. *et al. Nat. Genet.* **44**, 852–860 (2012).
7. Verma, A., Warner, S.L., Vankayalapati, H., Bearss, D.J. & Sharma, S. *Mol. Cancer Ther.* **10**, 1763–1773 (2011).
8. Linger, R.M., Keating, A.K., Earp, H.S. & Graham, D.K. *Expert Opin. Ther. Targets* **14**, 1073–1090 (2010).
9. Ye, X. *et al. Oncogene* **29**, 5254–5264 (2010).
10. Imai, K. & Takaoka, A. *Nat. Rev. Cancer* **6**, 714–727 (2006).
11. Jackman, D. *et al. J. Clin. Oncol.* **28**, 357–360 (2010).
12. Chaft, J.E. *et al. Clin. Cancer Res.* **17**, 6298–6303 (2011).

Genome stability, progressive kidney failure and aging

Hannes Lans & Jan H J Hoeijmakers

Two new studies report mutations in *FAN1* and three other genome-stability genes that tie the DNA damage response to progressive kidney failure and the dysfunction of several other organs. These findings provide clues to the underlying causes of tissue decline and may add a series of genes to the growing list of genome maintenance factors that protect against premature aging.

Karyomegalic interstitial nephritis (KIN) is a rare progressive renal failure disorder characterized by degeneration of renal tubules, interstitial fibrosis and typical polyploid nuclei (karyomegaly) in the kidney and often involves other tissues as well, including the brain and liver[1]. On page 910 of this issue[2], Friedhelm Hildebrandt, Agata Smogorzewska and colleagues report mutations in the *FAN1* gene, encoding Fanconi anemia–associated nuclease 1, as the cause of KIN in at least nine families. Because *FAN1* is involved in DNA damage repair, this discovery links the accumulation of DNA damage with chronic kidney failure.

Hannes Lans and Jan H.J. Hoeijmakers are in the Department of Genetics, Centre for Biomedical Genetics and Cancer Genomics Centre, Erasmus Medical Centre, Rotterdam, The Netherlands.
e-mail: j.hoeijmakers@erasmusmc.nl

FAN1 and kidney failure

FAN1 was only recently discovered[3–6] as a new DNA repair nuclease involved in the repair of highly cytotoxic DNA interstrand cross-links (ICLs), which prevent strand separation during mitosis. Although its exact role in this highly

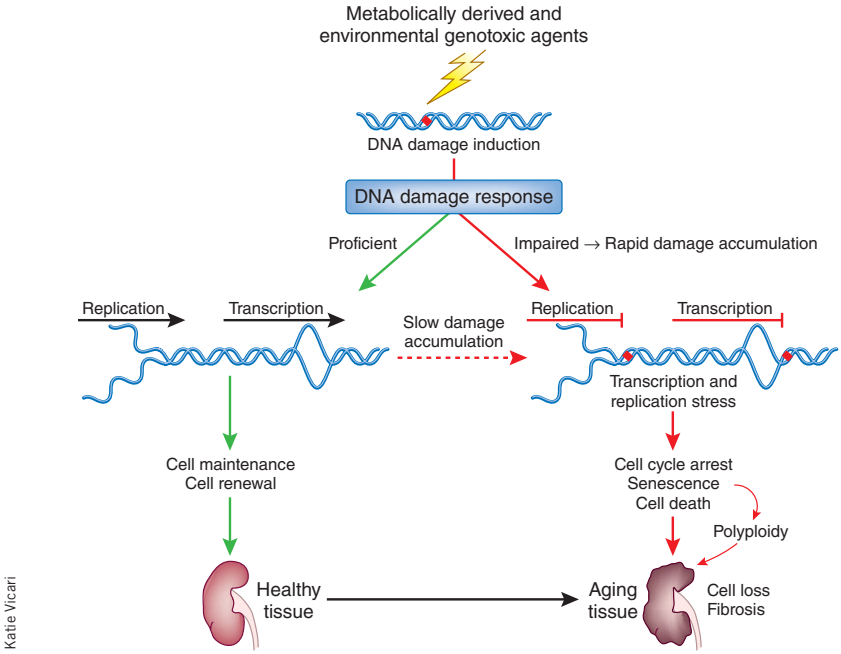

Figure 1 Links between DNA damage and aging. In healthy cells, an elaborate network of DNA repair and signaling pathways efficiently handles DNA damage that is continuously induced. Sporadic unrepaired damage accumulates slowly and contributes to aging. In cells that are defective in the DNA damage response, however, damage accumulates rapidly and leads to cell cycle arrest or death, causing premature aging.

complex process is still poorly defined, FAN1 has been suggested by several reports to function in the Fanconi anemia DNA repair pathway[3–6]. Fanconi anemia is a rare recessive disorder that illustrates various consequences of DNA damage, including highly variable developmental abnormalities related to damage-induced stochastic loss of important progenitor cells, progressive pancytopenic bone marrow failure that is likely due to early depletion of stem cells and cancer predisposition, mainly for acute myeloid leukemia, that results from damaged cells escaping death. Fanconi anemia is caused by mutations in one of at least 15 FANC family genes, whose products cooperate to remove ICLs from DNA. FAN1 localizes to sites of DNA damage by interacting with a FANC protein complex and is required for cell survival in response to ICL DNA damage. It is hypothesized that FAN1 may be the long-sought-after nuclease of the Fanconi anemia pathway that cleaves cross-linked DNA so that blocked replication can continue.

Notably, although FAN1-deficient cells from individuals with KIN show hypersensitivity to ICL-inducing agents[2], current evidence suggests that *FAN1* is not a typical susceptibility gene for Fanconi anemia. Despite the presence of some overlapping symptoms, the clinical features of individuals with KIN differ from those of individuals with Fanconi anemia. Furthermore, FAN1-deficient cells in KIN seem to behave differently from Fanconi anemia cells: they are less hypersensitive to ICL-inducing agents and score negative in a diagnostic test for Fanconi anemia[2,7]. Importantly, in FAN1-defective DT40 chicken cells as well as in FAN1-deficient human cells from KIN, additional loss of FANC proteins increases sensitivity to ICL-inducing agents[2,8]. Together, these observations suggest that FAN1 has other DNA repair functions in addition to an auxiliary role in the canonical Fanconi anemia pathway or that it is only involved in one branch of the process or in repair of a specific subset of ICL damage.

Zhou *et al.*[2] disrupted FAN1 function in zebrafish, which recapitulated KIN-like phenotypes, such as body axis curvature and the formation of renal cysts, confirming the importance of this nuclease and DNA damage repair to kidney function. They also observed that kidney failure was correlated with increased DNA damage signaling in a rat model and in human biopsies from damaged kidney transplants. Furthermore, in a parallel discovery reported in *Cell*, Friedhelm Hildebrandt and colleagues[9] identified mutations in three additional DNA damage response genes causing nephronophthisis-related kidney diseases, including Joubert and Senior-Loken syndromes (**Supplementary Table 1**).

These findings raise the question of how DNA repair defects lead to progressive kidney failure. Of course, DNA damage response proteins may have an unanticipated renal role in addition to their regular function in genome maintenance. However, many (geno)toxic agents induce renal failure. Moreover, KIN and polyploidization have been associated with exposure to high levels of environmental toxins or chemotherapeutics that damage renal cells[10,11]. Therefore, progressive kidney failure in FAN1-deficient individuals with KIN may similarly be induced by defects in genome maintenance causing cellular damage (**Fig. 1**). Cells unavoidably sustain DNA damage from exogenous sources but also from their own metabolism, which produces reactive species. In particular, an organ like the kidney, which continuously filters blood and transports waste chemicals, is exposed to high levels of toxic agents, which may induce DNA damage, including highly cytotoxic ICLs. DNA damage hampers vital processes, such as transcription and replication. If not properly repaired, as in FAN1-deficient KIN cells, ICLs will accumulate, leading to cell cycle arrest, senescence and cell death. In kidney and other tissues from individuals with KIN, DNA replication may occur but cannot be completed due to unrepaired damage, thereby preventing mitosis. A subsequent round of replication will lead to the characteristic aging marker of polyploidization or karyomegaly. The slow and late onset of KIN—affected individuals are all adults—is in line with the gradual accumulation of damage to the genome being the underlying cause. Loss of cell function and viability triggers fibrosis and progressive functional decline. In essence, the kidney ages prematurely.

Genome maintenance syndromes

Besides KIN-like features, *fan1* knockdown in zebrafish induced developmental defects that are frequently seen in association with other defects in genome maintenance pathways. Interestingly, four persons carrying a homozygous microdeletion spanning *FAN1* were recently described[7]. These individuals, who in contrast to individuals with KIN harbor a complete deletion of *FAN1*, show more severe developmental and degenerative characteristics, including microcephaly and many progressive neurodevelopmental abnormalities. No kidney problems were reported, likely because the affected individuals were still too young. Hence, it seems that the more severe the repair defect, the more severely and earlier the clinical features emerge.

Genetic defects in genome maintenance in humans and mice often lead to segmental progeroid features, meaning that many but not all organs age prematurely due to sustained DNA damage[12]. Notably, hallmarks of KIN—polyploidization and renal insufficiency—are characteristics of aging and are also observed in some other genome maintenance syndromes and in corresponding mouse models. Polyploidization is even more prominent in aging liver, which is the other organ

subjected to high levels of (geno)toxic compounds. Humans and mice deficient for the ERCC1-XPF endonuclease complex, which functions in the repair of intrastrand and ICL DNA damage, show extreme polyploidization in liver and kidney and have renal insufficiency[13–15]. Interestingly, liver abnormalities are also frequently observed in persons with KIN[1,2]. Thus, KIN with FAN1 deficiency likely constitutes another member of the growing list of genome maintenance syndromes that have a segmental and premature appearance of selected aging features (**Supplementary Table 1**). These findings link specific genome maintenance deficiencies to distinctive premature aging symptoms at the level of individual organs and tissues, which are likely due to a tissue-specific spectrum of DNA lesions caused by the unique metabolic profile of each particular organ or tissue.

Note: Supplementary information is available in the online version of the paper.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Palmer, D. *et al. Diagn. Cytopathol.* **35**, 179–182 (2007).
2. Zhou, W. *et al. Nat. Genet.* **44**, 910–915 (2012).
3. Kratz, K. *et al. Cell* **142**, 77–88 (2010).
4. Liu, T. *et al. Science* **329**, 693–696 (2010).
5. MacKay, C. *et al. Cell* **142**, 65–76 (2010).
6. Smogorzewska, A. *et al. Mol. Cell* **39**, 36–47 (2010).
7. Trujillo, J.P. *et al. Blood* published online 18 May 2012; doi:blood-2012-04-420604.
8. Yoshikiyo, K. *et al. Proc. Natl. Acad. Sci. USA* **107**, 21553–21557 (2010).
9. Chaki, M. *et al. Cell* (in the press).
10. Godin, M. *et al. Am. J. Kidney Dis.* **27**, 166 (1996).
11. McCulloch, T. *et al. Pediatr. Nephrol.* **26**, 1163–1166 (2011).
12. Garinis, G.A. *et al. Nat. Cell Biol.* **10**, 1241–1247 (2008).
13. Niedernhofer, L.J. *et al. Nature* **444**, 1038–1043 (2006).
14. McWhir, J. *et al. Nat. Genet.* **5**, 217–224 (1993).
15. Selfridge, J. *et al. Nucleic Acids Res.* **29**, 4541–4550 (2001).

The paradoxical TGF-β vasculopathies

Rosemary J Akhurst

Two new studies show that haploinsufficiency for *TGFB2* causes a familial syndrome of thoracic aortic aneurysms and dissections with other clinical features that overlap the Marfan, Loeys-Dietz spectrum of syndromes. Their finding of loss-of-function mutations in yet another transforming growth factor (TGF)-β pathway gene reinforces the seeming paradox of observed increases in the downstream TGF-β signaling pathway.

Thoracic aortic aneurysms (dilations) and dissections (tears) (TAADs) are a common cause of sudden death of young adults. Intracranial aneurysms and subarachnoid hemorrhage can also afflict seemingly healthy young adults, causing sudden mortality or severe morbidity. Such cardiovascular manifestations are often inherited in an autosomal dominant manner and can occur independently or as part of a syndrome, such as Marfan syndrome, Loeys-Dietz syndrome, arterial tortuosity syndrome and aneurysms-osteoarthritis syndrome. The aortic features of the Marfan, Loeys-Dietz (MLD) spectrum of disorders share clinical commonality in dilation of the aorta root, dysfunctional smooth muscle cells within the tunica media, with fragmentation and loss of elastic fibers, and excessive elaboration of extracellular matrix. Another common feature is paradoxical activation of the TGF-β signaling pathway in aortic lesions *in vivo*, despite the presence of what seem to be loss-of-function mutations in TGF-β signaling pathway components.

Rosemary J. Akhurst is at the Helen Diller Family Comprehensive Cancer Center, Department of Anatomy and Institute of Human Genetics, University of California at San Francisco, San Francisco, California, USA.
e-mail: rakhurst@cc.ucsf.edu

TGFB2 loss elevates TGF-β signaling

Two papers by Dianna Milewicz and colleagues and Bart Loeys and colleagues in this issue report that haploinsufficient loss-of-function mutations in a gene encoding TGF-β ligand, *TGFB2*, cause a novel syndromic form of TAAD[1,2]. This is an important finding because there has been considerable controversy surrounding the MLD syndromes, including discussion of whether decreased or elevated TGF-β signaling drives aortic aneurysm and other clinical manifestations[3]. The current finding is yet another example of loss of function of a TGF-β signaling component ultimately leading to a seemingly paradoxical increase in downstream signaling *in vivo*.

Two independent groups screened familial cases of thoracic aortic disease that could not be accounted for by mutations in the known causative genes *FBN1*, *TGFBR1*, *TGFBR2* and *SMAD3*. Boileau *et al.*[1] used 50K SNP genetic linkage analysis of two large families to map the locus to 1q41 around *TGFB2*, followed by exome sequencing of linked genes. Lindsay *et al.*[2] used a higher density (220K) SNP array analysis of severe probands from two families without previous genetic linkage analysis, and fortuitously found microdeletions (3.5 and 6.5 Mb) around *TGFB2*. Both groups found deleterious mutations in *TGFB2* that were also observed in affected family members but were not found in thousands of unrelated and unaffected individuals. Altogether, 12 independent mutations were identified, of which 8 were whole-gene deletions, frameshifts or nonsense mutations that are predicted to cause degradation of the cognate mRNA by nonsense-mediated decay, thereby indicating that the mutations cause loss of function. These mutations accounted for 1.5% (in ref. 1) and 25% (in ref. 2) of sampled familial cases of thoracic aortic disease that were not attributed to other known TAAD-causing genes. As with previous studies on what are known as 'TGF-β vasculopathies', despite causing genetic loss of function, mutations in both studies resulted in a paradoxical, although late, activation of the TGF-β signaling pathway, as shown by unequivocal elevation of the levels of phosphorylated SMAD2 and SMAD3 (SMAD2/3) in aortic lesions from *TGFB2*$^{+/-}$ persons and *Tgfb2*$^{+/-}$ mice, as well as by elevated ligand levels of either TGF-β1 (ref. 2) or TGF-β2 (ref. 1).

The canonical TGF-β signaling pathway[4] (**Fig. 1**) requires ligand binding to a heteromeric complex of type 1 and 2 serine/threonine kinase receptors. The TβRI receptor directly phosphorylates SMAD2/3, which then bind SMAD4 to accumulate in the nucleus where the complex transcriptionally activates many target genes, including the autoinductive *TGFB1* gene, the negative regulator *SMAD7* and the profibrotic factor *CTGF*. The three TGF-β ligands are synthesized as large latent forms that bind to extracellular matrix components,