bone-specific metastasis. The importance of these data, in our opinion, lies not with the magnitude of their prognostic significance. Breast cancer is now replete with prognostic gene signatures, from young women, primary tumors versus metastases, classes of tumors, wound signatures, etc. (Chang et al., 2005; Sorlie et al., 2001; van 't Veer et al., 2003). These data confirm the relevance of the lung-specific genes identified in a single model system to the heterogeneity of human disease.

Using microarray expression analysis of primary tumors from 82 patients from Memorial Sloan-Kettering Cancer Center, the predictive value of each of the 54 genes within their experimentally derived signature was determined using a Cox proportional hazards regression model. Relatively few of the functional lung-specific genes were significant. Of the genes modulating lung-specific metastasis as single transfectants, the p values were all nonsignificant (0.569–0.833). Of the genes analyzed in combination experiments, *MMP1* and *CXCL1* retained lung-specific prognostic significance, but others, including *COX2*, *SPARC*, and *EREG*, did not. Put simply, the functional genes were not the most

prognostic genes. It will be of interest to determine whether the most highly prognostic genes, such as latent TGF-β binding protein *LTBP1*, the Fascin homolog *FSCN1*, and the angiopoietin-like protein *ANGPTL4*, are functionally involved in lung-specific metastasis.

Minn et al. identified several potential players in the process of breast cancer metastasis to lung, but a detailed mechanism of organ-specific homing and colonization has yet to be established. The identification and validation of organ-specific metastatic pathways should lead to targeted therapeutics for these devastating diseases.

Christine E. Horak[1] and Patricia S. Steeg[1,*]

[1]Laboratory of Molecular Pharmacology
Center for Cancer Research
National Cancer Institute
Building 37, Room 1122
Bethesda, Maryland 20892
*E-mail: steegp@mail.nih.gov

Selected reading

Chang, H.Y., Nuyten, D.S., Sneddon, J.B., Hastie, T., Tibshirani, R., Sorlie, T., Dai, H., He,

Y.D., van 't Veer, L.J., Bartelink, H., et al. (2005). *102*, 3738–3743.

Fidler, I.J., and Kripke, M.L. (1977). Science *197*, 893–895.

Kang, Y., Siegel, P., Shu, W., Drobnjak, M., Kakonen, S., Corcon-Cardo, C., Guise, T., and Massague, J. (2003). Cancer Cell *3*, 537–549.

Lee, H., Lin, E.C., Liu, L., and Smith, J.W. (2003). Int. J. Cancer *107*, 528–534.

Minn, A.J., Gupta, G.P., Siegel, P.M., Bos, P.D., Shu, W., Giri, D.D., Viale, A., Olshen, A.B., Gerald, W.L., and Massague, J. (2005). Nature *436*, 518–524.

Montel, V., Huang, T.Y., Mose, E., Pestonjamasp, K., and Tarin, D. (2005). Am. J. Pathol. *166*, 1565–1579.

Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N., et al. (2001). Nature *410*, 50–56.

Paget, S. (1889). Lancet *1*, 571–573.

Sorlie, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 10869–10874.

Steeg, P. (2003). Nat. Rev. Cancer *3*, 55–63.

van 't Veer, L.J., Dai, H., van de Vijver, M.J., He, Y.D., Hart, A.A., Bernards, R., and Friend, S.H. (2003). Breast Cancer Res. *5*, 57–58.

Oh what a tangled web it weaves: BRCA1 and DNA decatenation

BRCA1 has significant roles in DNA repair and cell cycle checkpoint control, and is important in the maintenance of genomic stability. Defects in these pathways likely underpin the cancer susceptibility of *BRCA1* mutation carriers. Now, a new function for BRCA1 in DNA decatenation—removing the tangles introduced into chromosomes as a consequence of DNA replication—is suggested in a new paper by Lou et al. (2005) in *Nature Structural and Molecular Biology*. Ineffective DNA decatenation may lead to chromosome breakage and inappropriate repair, adding to the roll call of defects in *BRCA1* mutant cells.

A series of complex and orchestrated changes in chromosome structure are required to ensure the proper segregation of genetic material during cell division. A direct consequence of the double helical structure of DNA is that, after DNA replication during S phase, duplicated sister DNAs become topologically entangled or catenated (Wang, 2002). Sister chromatids continue to maintain a close association throughout G2 phase. Then, a signal at the onset of anaphase causes disruption of the linkage between sister chromatids, allowing them to be separat-

ed and pulled to opposite poles of the cell. However, a process called DNA decatenation needs to take place to separate chromosomes that have become entangled. This process involves DNA strand breakage and rejoining, and requires the enzyme Topoisomerase IIα (TopIIα) (Wang, 2002). Cells monitor the catenation of chromatids, and when these are insufficiently disentangled, the decatenation checkpoint is activated, arresting cells in metaphase (Deming et al., 2001). This checkpoint is separate from the response to DNA damage

(Skoufias et al., 2004) and may be inactivated in some cancers (Nakagawa et al., 2004), leading to inappropriate cell cycle progression, chromosome breakage, and genomic instability.

BRCA1 is a key regulator of DNA repair and the cell cycle in higher eukaryotic cells, and dysfunction leads to predisposition to breast and a variety of other cancers (Wooster and Weber, 2003). BRCA1 is required for the efficient repair of double-strand DNA breaks (DSBs) by homologous recombination, and BRCA1 deficiency leads to the uti-

lization of alternative DSB repair pathways, such as nonhomologous end joining (Turner et al., 2005). This is responsible, at least in part, for the genomic instability manifest in BRCA1 deficient cells. Additional functions for BRCA1 in cell cycle checkpoint regulation may also be relevant to carcinogenesis in *BRCA1* mutation carriers. BRCA1 is required for the DNA damage-induced G2 cell cycle checkpoint, and is also implicated in controlling the decatenation checkpoint along with ATR (Deming et al., 2001). Now, Junjie Chen and colleagues (Lou et al., 2005) provide evidence for a new role for BRCA1 in DNA decatenation itself that suggests a further mechanism whereby BRCA1 deficiency might contribute to genomic instability (Figure 1).

The starting point of the work of Lou et al. (2005) was the previous observation that both BRCA1 and TopIIα are present in nuclear foci in S phase of the cell cycle, and that some of these foci coincide. After confirming this observation, the hypothesis that BRCA1 and TopIIα proteins physically interact was tested by coimmunoprecipitation. This indicated that the proteins did indeed interact, directly or indirectly, and that the C terminus of BRCA1, encompassing the two tandemly arranged BRCT domains, was required for this interaction. The BRCT domains are phospho-Serine or -Threonine binding motifs, which mediate protein-protein interactions (Yu et al., 2003; Manke et al., 2003). In agreement with the specificity of BRCT domains, BRCA1 only bound TopIIα that was phosphorylated. However, it is unclear which residue(s) within TopIIα need to be phosphorylated to mediate the interaction with BRCA1, nor which kinase is responsible for phosphorylating this residue. The issue of whether the proteins interact directly or indirectly via bridging proteins also needs to be resolved.

Chromosome decatenation, which is necessary before chromosomes can condense and segregate, requires TopIIα activity; treatment of cells with a TopIIα chemical inhibitor, ICRF-193, results in a defect in chromosome segregation which manifests as the presence of bridge-like structures at mitosis con-
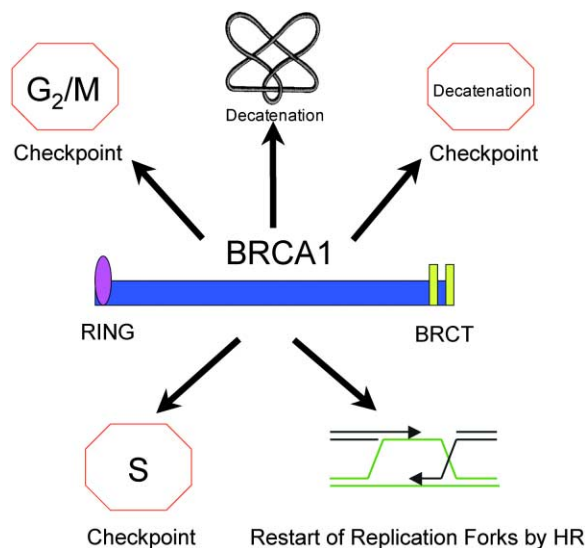

Figure 1. Cell cycle-related functions of BRCA1

Double strand DNA breaks or arrested or collapsed replication forks occurring in S phase are repaired in part by BRCA1-dependent homologous recombination (Turner et al., 2005). BRCA1 acts in the intra-S and G2/M phase cell cycle checkpoints (Xu et al., 2001). BRCA1 is required for efficient decatenation of chromatids (Lou et al., 2005) and the checkpoint which monitors whether this has occurred (Deming et al., 2001).

taining lagging chromosomes. Similarly, in cells that are deficient for BRCA1, either by mutation or depletion using siRNA, lagging chromosomes are frequently observed. Importantly, BRCA1 deficiency did not induce any alterations in cell cycle profile, nor was the mitotic checkpoint impaired. Consistent with BRCA1 modulating TopIIα function, elongated (noncondensed) and tangled chromosomes were observed in BRCA1-deficient cells, mirroring the effects of TopIIα inhibition by ICRF-193. This suggested a role for BRCA1 in DNA decatenation. In vitro assays have been developed to measure DNA decatenation activity in nuclear extracts using kinetoplast DNA as a substrate. This activity, present in nuclear extracts, appears to be dependent on TopIIα, as it is inhibited by ICRF-193. Therefore, nuclear extracts were prepared from BRCA1-deficient and complemented cell lines, and these assayed for the ability to decatenate DNA. This revealed that BRCA1 was indeed required for the in vitro decatenation activity present in nuclear extracts.

One obvious way in which BRCA1 might affect the ability to decatenate DNA was by altering levels of TopIIα protein, but this appears not to be the case.

TopIIα phosphorylation has been recognized previously as having a role in the regulation of TopIIα activity, and modulation of phosphorylation was investigated as a possible mechanism. A gross change in TopIIα phosphorylation state modulated by BRCA1 was assayed by determining the level of interaction with the MPM-2 protein, which only binds to phosphorylated TopIIα. This was apparently unaltered, but subtle alterations in phosphorylation state might not be recognized using this method. Given the established role of BRCA1 in mediating phosphorylation of proteins by upstream kinases in response to DNA damage (Foray et al., 2003), this possibility requires further investigation. BRCA1, in association with a heterodimeric partner BARD1, has ubiquitin ligase activity, but direct substrates of this activity have been difficult to identify (Kerr and Ashworth, 2001). Given that BRCA1 is associated in cells with TopIIα, Lou et al. examined whether TopIIα was modified by ubiquitination in a BRCA1-dependent fashion. In normal cells, TopIIα was apparently modified by ubiquitin conjugation, but this was reduced in BRCA1-deficient cells. Ubiquitination conjugation can affect protein degradation or modify function or subcellular location; in this case, conjugation did not seem to affect the stability of TopIIα.

So what might be the effects of BRCA1-mediated ubiquitination of TopIIα? The possibility was explored that this altered the subnuclear location of the TopIIα and in particular association of the protein with chromatin. Unmodified TopIIα was more tightly associated with chromatin than the ubiquitinated form, and chromatin associated TopIIα was less enzymatically active in the decatenation assay described above. Therefore, there appears to be a correlation between BRCA1-dependent TopIIα ubiquitination and TopIIα activity, the implication being that BRCA1 deficiency reduces TopIIα activity, and this induces the decatenation defect.

Despite providing intriguing insight into a new way in which BRCA1 deficiency can induce genomic instability, several questions remain, particularly relating to the exact role of BRCA1 in decatenation.

First, does the BRCA1/BARD1 heterodimer directly ubiquitinate TopIIα? Second, does ubiquitination of TopIIα abrogate chromatin association, or does the protein simply become ubiquitinated when it becomes less tightly bound to chromatin? Finally, the defect in DNA repair by homologous recombination in BRCA1-deficient cells provides an avenue for potential novel therapeutic approaches (Farmer et al., 2005). It may be that the decatenation defect caused by BRCA1 deficiency may provide an additional route to specifically target tumors arising in BRCA1 mutation carriers.

Alan Ashworth[1,*]

[1]The Breakthrough Breast Cancer
 Research Centre
Institute of Cancer Research
Fulham Road
London SW3 6JB
United Kingdom
*E-mail: alan.ashworth@icr.ac.uk

Selected reading

Deming, P.B., Cistulli, C.A., Zhao, H., Graves, P.R., Piwnica-Worms, H., Paules, R.S., Downes, C.S., and Kaufmann, W.K. (2001). Proc. Natl. Acad. Sci. USA *98*, 12044–12049.

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., et al. (2005). Nature *434*, 917–921.

Foray, N., Marot, D., Gabriel, A., Randrianarison, V., Carr, A.M., Perricaudet, M., Ashworth, A., and Jeggo, P. (2003). EMBO J. *22*, 2860–2871.

Kerr, P., and Ashworth, A. (2001). Curr. Biol. *11*, R668–R676.

Lou, Z., Minter-Dykhouse, K., and Chen, J. (2005). Nat. Struct. Mol. Biol. *12*, 589–593.

Manke, I.A., Lowery, D.M., Nguyen, A., and Yaffe, M.B. (2003). Science *302*, 636–639.

Nakagawa, T., Hayashita, Y., Maeno, K., Masuda, A., Sugito, N., Osada, H., Yanagisawa, K., Ebi, H., Shimokata, K., and Takahashi, T. (2004). Cancer Res. *64*, 4826–4832.

Skoufias, D.A., Lacroix, F.B., Andreassen, P.R., Wilson, L., and Margolis, R.L. (2004). Mol. Cell *15*, 977–990.

Turner, N., Tutt, A., and Ashworth, A. (2005). Curr. Opin. Pharmacol. *5*, 388–393.

Wang, J.C. (2002). Nat. Rev. Mol. Cell Biol. *3*, 430–440.

Wooster, R., and Weber, B.L. (2003). N. Engl. J. Med. *348*, 2339–2347.

Xu, B., Kim, S., and Kastan, M.B. (2001). Mol. Cell. Biol. *21*, 3445–3450.

Yu, X., Chini, C.C., He, M., Mer, G., and Chen, J. (2003). Science *302*, 639–642.