The requirement for the maintenance of telomeres by telomerase by most cancer cells for continued proliferation is a target in anticancer strategies. Tankyrases are poly(ADP-ribose) polymerases that enhance telomerase access to telomeres. Tankyrase 1 modulates telomerase inhibition in human cancer cells and is reviewed in this report as a potential telomere-directed anticancer target.

Keywords: tankyrase; poly(ADP-ribosyl)ation; telomeres; telomerase

TELOMERE ELONGATION BY TANKYRASE 1

In telomerase-positive cells, average telomere length is stabilized by a 'protein-counting' mechanism, in which a series of telomere-associated proteins negatively regulate telomere elongation by telomerase (reviewed in Smogorzewska and de Lange, 2004). Longer telomeres have greater numbers of TRF1, a double-stranded telomeric repeat binding protein. In conjunction with the downstream TIN2-TPP1-POT1 telomere-associated complex, TRF1 blocks access of telomerase to telomeres (Figure 2).

Tankyrase 1 (TRF1-interacting ankyrin-related ADP-ribose polymerase 1) was originally identified as a TRF1-binding protein by using a yeast two-hybrid screen (Smith et al, 1998). This 140-kDa protein consists of four characteristic domains (Figure 1): the N-terminus is known as the HPS domain, containing homopolymeric runs of histidine, proline, and serine. The functional significance of the HPS domain is unknown. Tankyrase 1 is also comprised of an ANK domain placing the protein within the ANK family of proteins. As in the original ankyrins, the tankyrase 1 ANK domain is composed of a long stretch of 24 ANK repeats, providing a platform for protein–protein interactions. Unlike the ANK of ankyrins, the ANK domain of tankyrase 1 is further divided into five well-conserved subdomains (Seimiya and Smith, 2002; De Rycker et al, 2003). Each subdomain, designated as ARC (ANK repeat cluster) I–V, works as an independent TRF1-binding site. TRF1 recognition by the most C-terminal subdomain ARC V is the most important for the telomeric function of tankyrase 1 (Seimiya and Smith, 2002; Seimiya et al, 2004). The sterile alpha motif (SAM) domain, adjacent to the ARC V domain, contributes to multimerization of tankyrase 1 (De Rycker et al, 2003; De Rycker and Price, 2004). The most striking feature of tankyrase 1 is the C-terminal PARP domain, which catalyses poly(ADP-ribosylation) of acceptor proteins using NAD as a substrate. This post-translational modification provides significant negative charges to the acceptor proteins and often disrupts interactions between the acceptor proteins and the DNA. In general, poly(ADP-ribosylation) is involved in various physiological events, such as DNA replication, DNA repair, gene expression, chromatin decondensation, malignant transformation, cellular differentiation, and apoptosis.
Tankyrase 1 recognizes TRF1 via the ARC subdomains within the ANK domain and is localized at the telomeres (Smith et al., 1998; Cook et al., 2002; Seimiya and Smith, 2002). TRF1 is poly(ADP-ribosyl)ated by tankyrase 1, and this modification blocks the ability of TRF1 to bind telomeric DNA in vitro (Smith et al., 1998). In intact human cells, tankyrase 1 releases TRF1 from telomeres in a PARP activity-dependent manner (Smith and de Lange, 1999; Cook et al., 2002). Released TRF1 is rapidly ubiquitinated and subjected to proteasomal degradation (Chang et al., 2003), thereby explaining the striking linkage between poly(ADP-ribosyl)ation and protein degradation. Consistent with these observations, telomere elongation is induced by the overexpression of exogenous tankyrase 1 in the nucleus of telomerase-positive cells (Smith and de Lange, 2000). Also confirming the above findings, tankyrase 1-mediated telomere elongation is not observed in the absence of telomerase activity (Cook et al., 2002; Chang et al., 2003; Seimiya et al., 2005). Thus, by enhancing telomere access to telomerase, tankyrase 1 works as a positive
regulator for telomere elongation by telomerase. This leads to the supposition that the action of telomerase at the telomere may be regulated by inhibitors of telomerase and also by molecules that regulate tankyrase 1, and hence telomere structure and telomerase access to the telomere (see below).

Tankyrase 2 is a closely related homologue of tankyrase 1 (see below). Tankyrase 1 forms a ternary complex with TRF1 and another TRF1-binding protein, TIN2. In this complex, poly(ADP-ribosyl)ation of TRF1 is prevented by TIN2 (Ye and de Lange, 2004). The mechanisms underlying tankyrase activation or inhibition are unknown. It is known that tankyrase 1 is not activated by damaged DNA, which is an activator of PARP-1, the most abundant member among the PARP family (Cook et al, 2002).

TANKYRASES AS TARGETS FOR CANCER THERAPY

As described above, telomere maintenance by telomerase is the Achilles’ heel of infinite growth for most cancer cells (Hahn et al, 1999; Zhang et al, 1999). Continuous treatment of cancer cells with telomerase inhibitors shortens telomeres and eventually induces cellular senescence and/or apoptosis (Seimiya et al, 2002 and references therein). Thus, according to this simple scenario, telomerase inhibitors have the potential to benefit cancer patients in the future.

A potential disadvantage is that telomere shortening depends on the repetitive occurrence of the DNA end replication problem resulting from cell division. For this reason, it is essential that telomerase inhibitors are not cytotoxic. Furthermore, as telomere loss is a gradual process there is a lag between the time telomerase is inhibited and the time telomeres shorten sufficiently to disrupt the capping function. This would necessitate long treatment schedules that may lead to acquired drug resistance both in the cell and throughout the body. In general, longer telomeres provide more binding sites for TRF1, which blocks telomere access to telomerase. Accordingly, telomere shortening per se compromises the ability of telomerase inhibitors since shorter telomeres have fewer TRF1 molecules and therefore allow easier access to residual telomerase activity (Seimiya et al, 2005). Thus, the rate of telomere shortening per cell division decreases with telomere shortening itself. This phenomenon results from the incomplete shutdown of telomerase activity by telomerase inhibitors. A better therapeutic outcome may result from increasing the efficiency of telomere shortening to hasten the telomere crisis.

Telomere accessibility is also a potential target for telomerase inhibition. Inhibition of tankyrase, that enhance telomere access to telomeres, may indirectly induce cancer cell senescence by abrogating telomerase activity. Support for this rational is provided by the finding that tankyrase 1 confers resistance to telomerase inhibitors (Figure 2). In these experiments cells overexpressing tankyrase 1, which removes TRF1 from telomere DNA, have unchanged telomere length following treatment with the telomerase inhibitor, MST-312. This drug resistance is reversed by several known PARP inhibitors, such as 3-aminobenzamide (3AB) and PJ-34, which are able to block tankyrase 1 PARP activity (Seimiya et al, 2005). Even in cells that do not overexpress exogenous tankyrase 1 (but do express endogenous tankyrase 1), these PARP inhibitors enhance the rate of telomere shortening by means of a telomerase inhibitor, MST-312 (Seimiya et al, 2002), and induce earlier cell crisis. These PARP inhibitors do not directly inhibit telomerase activity but lead to telomere shortening, to a small extent, presumably by reducing telomere access to telomerase. Furthermore, MST-312 resistance caused by telomere shortening per se is reversed by 3AB. By comparison, 3AB has no effect on telomere length in telomerase-independent ALT (alternative lengthening of telomeres)-type cells, which maintain their telomere length by DNA recombination. Also, telomere shortening caused by an inherent end replication problem in normal fibroblasts is not accelerated by 3AB (Seimiya et al, 2005). Thus, it is expected that the effect of such PARP inhibitors on telomere length is selective to telomerase-positive cells. These observations provide support for tankyrase 1 as a suitable target for telomere-directed cancer therapy. Pathologically, tankyrase 1 gene expression is elevated in some tumours but not in others (Gelmini et al, 2004 and references in therein).

Another avenue for telomerase inhibition has recently emerged. Li et al (2004) reported that knockdown of the hTR telomerase RNA component by RNA interference (RNAi) induces a rapid antiproliferative effect on telomerase-positive cancer cells. Unexpectedly, this effect occurs without telomere attrition and is thereby independent of the initial telomere length of the target cell. These observations suggest that telomerase inhibitors may exert a more acute therapeutic effect than expected.

OTHER FACES OF TANKYRASES

Multiple functions of tankyrases in accordance with a variety of binding partners pose the next challenging question about potential side effects of tankyrase-directed cancer therapy. Tankyrase 1 is also present at nontelomeric loci, including mitotic centrosomes, nuclear pore complexes, and Golgi apparatus (Smith and de Lange, 1999; Chi and Lodish, 2000). Furthermore, tankyrase 1 has a closely related homologue, tankyrase 2 that unlike tankyrase 1 lacks HPS domain. Tankyrase 1 is relatively abundant in reproductive tissues (i.e. testis and ovary), whereas the expression of tankyrase 2 is ubiquitous (Smith et al, 1998; Kaminker et al, 2001; Lyons et al, 2001; Cook et al, 2002). The functional difference and redundancy between the two proteins remain unknown.

Nontelomeric tankyrase 1/2-binding partners include insulin-responsive aminopeptidase (IRAP) (Chi and Lodish, 2000), the Grb14 signalling adaptor protein (Lyons et al, 2001), the 182 kDa tankyrase-binding protein (TABI82) (Seimiya and Smith, 2002), the nuclear/mitotic apparatus protein (NuMA) (Sbodio and Chi, 2002; Chang et al, 2005b), the Mcl-1 apoptotic regulator (Baे et al, 2003), and the Epstein – Barr virus nuclear antigen-1 (EBNA-1) (Deng et al, 2005). So far, TRF1, IRAP, TABI82, NuMA, EBNA-1 and tankyrase 1 and 2 are poly(ADP-ribosyl)ated by tankyrases. The Golgi tankyrase 1 colocalizes with the glucose transporter GLUT4 vesicles where tankyrase 1 is associated with IRAP (Chi and Lodish, 2000). In insulin-stimulated adipocytes, tankyrase 1 is phosphorylated at serine residues by the mitogen-activated protein kinase pathway. Phosphorylation of tankyrase 1 results in upregulation of its intrinsic PARP activity (Chi and Lodish, 2000). Although the function of tankyrase 1 at the Golgi is unclear, the artificial formation of tankyrase 1-containing vesicles disrupts Golgi structure and inhibits apical secretion (De Rycker and Price, 2004).

During mitosis, tankyrase 1 is concentrated around the pericentriolar matrices (Smith and de Lange, 1999) in a NuMA-dependent manner (Chang et al, 2005b). NuMA plays an essential role in organizing microtubules at the spindle poles. As NuMA is poly(ADP-ribosyl)ated by tankyrase 1 during mitosis (Chang et al, 2005b), it is possible that tankyrase 1 regulates NuMA’s function at the spindle poles. Interestingly, poly(ADP-ribosylation) is required for spindle assembly and structure (Chang et al, 2004), and tankyrase 1 is a key player in these processes (Chang et al, 2005a). Another fraction of tankyrase 1 remains at telomeres during mitosis (Smith et al, 1998) and is thought to play a role in sister chromatid resolution at telomeres. Support for this role of tankyrase 1 was provided by the metaphase arrest of cell division in tankyrase 1 knockdown experiments in which pairs of sister chromatids remain associated only at telomeres (Dynek and Smith, 2004). Recently, metaphase arrest by tankyrase 1 knockdown has been reported by another group, who shows intact sister
chromatid cohesion, instead of telomeric cohesion, in tankyrase 1
knockdown cells (Chang et al., 2005a).

The protein structure of tankyrases suggests they act as
scaffolding molecules. First, each of the five ARC subdomains
works as an independent recognition site for tankyrase-binding
proteins. This suggests that even a single tankyrase molecule can
interact with multiple binding partners (Seimiya and Smith, 2002;
Seimiya et al., 2004). Secondly, the SAM domain multimerizes
in tankyrases to an auto-poly(ADP-ribosyl)ation-sensitive manner.
This multimerization presumably leads to assembly of a larger
molecular lattice (De Rycker et al., 2003; De Rycker and Price,
2004) and may explain why tankyrase-binding proteins often
localize to higher order intracellular structures, such as telomeres
(TF1), Golgi (IRAP), spindle poles (NuMA), and cortical actin
(TAB1&2).

It is intriguing that murine TRF1 lacks the tankyrase recognition
consensus site, RXX(P/A)DG, suggesting that the telomeric
function of tankyrases is not conserved in mice (Sbodio and Chi,
2002). Other reported functions of tankyrases include involvement
in apoptosis (Bae et al., 2003) and episomal regulation of Epstein –
Barr virus OriP (origin of plasmid) (Deng et al., 2005). Taken
together, these observations suggest an expanding network of
tankyrase-mediated biological processes.

CONCLUDING REMARKS

The pharmacological targeting of tankyrase 1 is a potentially
significant anticancer strategy if used in conjunction with
inhibitors of telomerase. This trend would further promote
development not only of telomerase inhibitors but also of PARP
inhibitors. In fact, recently, PARP inhibitors have been shown to
be powerful against DNA repair-deficient tumours with the
advantage of low cytotoxicity (Bryant et al., 2005; Farmer et al.,
2005). PARP inhibitors are also implicated in other PARP-related
diseases, such as stroke, myocardial ischaemia, diabetes, and
central nervous system injury. Tankyrases also have multiple
functions and we have to consider the potential side effects
resulting from their inhibition. It is possible that the function of
tankyrase 1 in mitosis (Dynek and Smith, 2004; Chang et al., 2005a)
is less affected by PARP inhibitors than its function in
maintenance of telomere length as mitosis is unaffected in PARP
inhibitor-treated cells (Seimiya et al., 2005). At the physiological
level, mechanisms for tankyrase regulation and the functional
redundancy between tankyrase 1 and 2 are largely unknown.
Future experiments in tankyrase 1 and 2 knockout animals may
elucidate the mechanisms underlying the many functions of
the tankyrase enzymes.

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