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Review

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Noncanonical Functions of Telomerase: Implications in Telomerase-Targeted Cancer Therapies

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Abstract

Telomerase plays a pivotal role in bypassing cellular senescence and maintaining telomere homeostasis, essential properties required for the sustenance and progression of cancer. However, recent investigations have uncovered extratelomeric properties of telomerase that are independent of its role in telomere extension. This review summarizes recent insights to the noncanonical functions of telomerase reverse transcriptase (TERT) catalytic subunit, in particular in cancer progression, and highlights two major signaling mechanisms involved in the cross-talk with TERT—the NF-κB and Wnt/β-catenin pathways. We propose a feed-forward regulatory loop mechanism underlying TERT activation in cancers in which TERT acts as a transcriptional modulator of oncogenic signaling pathways that sustain its own levels and control the induction of target genes critical for tumor cell survival and proliferation. Finally, we provide a new perspective on telomerase-targeted cancer therapies and suggest possible interventions targeting the nontelomeric roles of TERT. This therapeutic strategy can be used in the future targeting of other telomerase components that exhibit novel nontelomeric functions in cancer and other ailments. *Cancer Res; 74(6); 1639–44. ©2014 AACR.*

Telomere-Associated Proteins Have Extratelomeric Roles in Regulating Diverse Signaling Pathways in Mammals

Telomeres are tandem repeats of the sequence TTAGGG found at chromosomal ends of eukaryotes. These nucleoprotein structures harbor binding sites for a group of telomeric proteins, which collectively constitute the shelterin complex that executes protective mechanisms against chromosomal degradation, end-to-end fusions and DNA-damage responses. The shelterin complex is composed of six members—telomeric repeat binding factor 1 (TRF1), TRF2, TRF1-interacting nuclear factor (TIN2), protection of telomeres 1, TIN2 organizing protein, and repressor-activator protein 1 (RAP1). Besides their role in capping chromosome ends, shelterin components have been demonstrated to regulate telomerase recruitment and activity at the telomere (1). Telomeres and telomeric proteins play a nonredundant role in maintenance of chromosomal stability and genomic integrity. However, extratelomeric roles of shelterin components have been uncovered by a number of recent studies, revealing a new perspective on telomeres as biologic signaling hubs in mammals. Teo and colleagues identified a cytoplasmic function of RAP1 in regulation of the NF-κB signaling cascade, which was distinct from its telomeric function (2). In particular, mammalian RAP1 was found to associate with the inhibitor of IκB kinases (IKK), enzymes that facilitate NF-κB activation by phosphorylating inhibitory IκBα proteins bound to NF-κB, thereby leading to their subsequent ubiquitylation and degradation (2). This interaction between RAP1 and IKKs in the cytosol was reported to be critical for recruiting IKK to the NF-κB p65 subunit, resulting in its phosphorylation and consequent transcriptional activation (2). The authors further implicated elevated cytoplasmic RAP1 levels in mediating constitutive IKK and hence NF-κB activity and function, which is correlated with invasive human breast cancers (2). In another independent study, Martinez and colleagues demonstrated the role of mammalian RAP1 in transcriptional regulation at extratelomeric sites genome-wide, similar to its ortholog in budding yeast *Saccharomyces cerevisiae* (3). Although RAP1-bound loci were identified at both telomeric and extratelomeric regions containing TTAGGG repeats via whole-genome chromatin immunoprecipitation sequencing (ChIP-seq), increased occupancy of extratelomeric RAP1-binding sites at subtelomeric regions correlated with preferential derepression of subtelomeric genes in RAP1-null cells, thereby supporting the nontelomeric role of RAP1 in transcriptional control (3). More recently, RAP1 was also found to regulate obesity through its involvement in signaling pathways connected to metabolism (4, 5). Till date, extratelomeric functions of other shelterin proteins besides RAP1 have also been described previously (1, 6, 7). Thus, collectively, evidence depicting the diverse functions of telomeric proteins in a myriad of signaling events at extratelomeric locations, reiterates the view that telomeres in mammals (much like in yeast) function as critical "signaling hubs" that can regulate fundamental physiologic processes governing aging, cancer, inflammation, and metabolism.

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Telomerase Extends Telomeres, Thereby Conferring Cellular Immortality

Telomerase is a ribonucleoprotein enzyme, which is well recognized for its role in *de novo* telomeric DNA synthesis. Its minimal catalytic core comprises of telomerase reverse transcriptase (TERT) and the RNA (TERC) components. TERT protein catalyzes the addition of telomeric repeats at chromosome ends using TERC as the template for extension. In addition to the minimal catalytic core, other proteins found to be associated with the telomerase complex include the RNA-binding protein Dyskerin, Reptin, Pontin, telomerase Cajal body protein 1, and several small ribonucleoproteins such as NHP2, NOP10, and GAR1 (8, 9). This multisubunit telomerase complex is essential for maintaining telomere homeostasis, which has been traditionally viewed as a central regulator of aging and cancer. This long-standing notion stems from past observations of the progressive shortening of telomeres following each cycle of DNA replication, owing to the inability of DNA polymerase to fully replicate chromosomal ends (10). Following telomere shortening to a critical length, telomere erosion and the consequential loss of telomere capping function occurs. This triggers a DNA-damage response that activates the p53 tumor suppressor pathway, thereby inducing replicative senescence in primary human cells (8). Thus, most somatic cells that possess limited telomere reserves due to negligible telomerase activity exhibit a finite replicative potential. This can be circumvented through reactivation of telomerase, which confers cellular immortalization (8). Besides its reverse transcriptase ability, nontelomeric functions have been ascribed to various protein components in the telomerase complex, including p53 biogenesis, DNA-damage repair, and chromatin remodeling (9). In this review, we will focus mainly on the noncanonical roles of the catalytic subunit, TERT, which contribute to cancer development and progression.

Telomerase Reactivation Is a Critical Step in Human Cancer Progression

In contrast with the constitutive expression of TERC and other components of the telomerase complex in adult somatic tissues and germ cells, TERT is normally present at low levels in human stem cells and germ cells, while being virtually undetectable in most adult somatic tissues due to transcriptional repression (11). However, during human tumorigenesis, telomerase is often reactivated via transcriptional upregulation of TERT. The expression of telomerase can be stimulated by transcription factors/coregulators that are well documented for their cancer-promoting roles, such as c-Myc (12), NF-κB (13) and β-catenin (14, 15). Telomerase upregulation enables rapidly proliferating cancer cells to bypass checkpoint signaling pathways that are activated during critical telomere shortening to induce cellular senescence and cell death. Hence, telomerase reactivation, which is found in nearly 90% of all advanced cancers (11), is instrumental in endowing tumor cells with an immortal growth potential that is believed to form a key step in the adenoma–carcinoma transition during human cancer development. In addition, as telomerase is constitutively expressed in stem cells, certain cancers are believed to arise from cancer stem cells with enhanced telomerase activity (16).

Although early studies have attributed the prime function of telomerase activation in cancer progression to its ability to extend telomeres, there is mounting evidence highlighting that additional activities of telomerase beyond telomeres could contribute profoundly to tumorigenesis. In a study conducted by Artandi and colleagues, which addressed past observations of enhanced telomerase activity during tumor development in laboratory mice despite them possessing long telomeres, transgenic mice expressing reverse transcriptase telomerase, mTERT, developed spontaneous neoplastic lesions in their mammary glands that progressed to invasive mammary carcinomas (17). Although TERT activation promoted mammary carcinogenesis in aging mice, mTERT overexpression in mouse embryonic fibroblasts could not overcome replicative senescence despite their extended telomeres, suggesting that mere telomere elongation does not account for the prooncogenic activity of telomerase. This ability of TERT to confer additional oncogenic capabilities, independent of its telomeric function, was further highlighted in an immortal human fibroblast cell line exhibiting alternative lengthening of telomeres (ALT). In a separate study by colleagues from the Weinberg laboratory, expression of oncogenic H-Ras in ALT cells was insufficient to confer tumorigenicity, whereas subsequent ectopic expression of hTERT or a catalytically active mutant form of hTERT that has lost the ability to elongate telomeres resulted in transformation (18). These data reinforce the view that telomerase performs "extracurricular" activities beyond its function in telomere stabilization, which promote carcinogenesis. Moreover, the ability of alternatively spliced variants of TERT, which lack telomerase activity, to induce the expression of growth-promoting genes, stimulate cell proliferation, and protect cancer cells from apoptosis provides further corroborating evidence to the noncanonical functions of telomerase in tumorigenesis (19–21). Interestingly, recent work by Mukherjee and colleagues, through ectopic expression of a panel of hTERT mutants in human mammary epithelial cells, demonstrated that functions of hTERT in cellular proliferation, lifespan extension, and DNA-damage signaling are each separable and distinct from its telomere elongation role (22). In addition, the authors showed that the catalytic activity of hTERT, independent of telomere elongation, was necessary for its proproliferative function (22). These findings support the earlier observations by Mukherjee and colleagues and provide further insight to the noncanonical functions of TERT. We shall discuss some of the mechanisms of action underscoring the noncanonical roles of telomerase in cancer-promoting events and suggest potential interventions for telomerase-targeted cancer therapies in the following sections.

Noncanonical Functions of Telomerase That Intersect with Signaling Pathways Controlling Development, Oncogenesis, and Inflammation

Several recent reports have documented the noncanonical functions of telomerase in signaling cascades that influence cancer development and progression, including the NF-κB and

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Wnt/β-catenin pathways. The transcription factor NF-κB is a key regulator of inflammatory and developmental processes in which deregulation has been implicated in the pathogenesis of various human ailments, including inflammation and cancer (23). Although the transcription factor has been documented to bind to the TERT promoter and activate its transcription (13), the first evidence for the direct interaction between NF-κB p65 subunit (RelA) and TERT protein was demonstrated in multiple myeloma cells in which p65 was found to modulate nuclear translocation of TERT (24). However, it was nearly a decade later before the mechanistic basis for the interaction between TERT and the NF-κB signaling pathway was illuminated (25). The authors showed that ectopic expression of telomerase resulted in increased cancer cell proliferation, protection from cell death as well as larger tumors in a xenograft model, which could be mitigated by dampening NF-κB signaling (25). Conversely, hTERT abrogation by siRNAs significantly repressed tumor growth, a phenomenon that could be reversed by p65 overexpression (25). These findings support a functional interplay between telomerase and NF-κB signaling, which was further reinforced by the observation that telomerase overexpression resulted in enhanced expression of NF-κB target genes, whereas telomerase-null mice were refractory to NF-κB activation. Intriguingly, TERT was found to bind to p65 and localize to promoters of a subset of NF-κB target genes, including *interleukin (IL)-6, TNF-α*, and *IL-8*—cytokines known to sustain inflammation and cancer progression (25). Moreover, inhibition of telomerase with MST-312 reduced the expression of *IL-6* in primary leukemic cells, further substantiating the role of telomerase as a modulator of NF-κB–dependent transcription in human cancers (25).

This noncanonical activity of telomerase in the NF-κB signaling pathway was also illustrated in a recent study by Ding and colleagues. In particular, the authors demonstrated that both hTERT and its catalytic mutant hTERT K626A, which lacks reverse transcriptase activity, could activate NF-κB–mediated transcription and regulate the expression of several NF-κB target genes to similar extents (26). Furthermore, overexpression of hTERT or hTERT K626A promoted the nuclear accumulation of NF-κB p65 as well as p65 DNA binding at both basal and TNF-α–induced states (26). These data reiterate the notion that telomerase can function as a transcriptional modulator of the NF-κB signaling cascade in a fashion distinct from its activity at telomeres. Moreover, the observation that NF-κB can serve as a transcriptional inducer of TERT and vice versa highlights the functional interconnectedness and likely positive feedback regulation between NF-κB and hTERT. This feed-forward regulatory loop may be one of the mechanisms underlying the concomitant chronic inflammation and prolonged telomerase activity apparent in human cancers.

Besides NF-κB, telomerase has also been reported to regulate the transcriptional activity of the Wnt/β-catenin complex. The Wnt/β-catenin signaling pathway is a central regulator of embryogenesis and the self-renewal property of adult stem cells in proliferating tissues such as the intestine, hair follicle, and hematopoietic system (27). Being one of the key mechanisms governing cell proliferation, cell polarity, and cell fate determination (27), deregulation of the Wnt/β-catenin signaling cascade is often connected to a variety of developmental disorders and cancers. The first connection between telomerase and the Wnt/β-catenin pathway was discovered in hair follicle stem cells in which transgenic mice engineered to conditionally express catalytically inactive TERT ($TERT^{ci}$) in keratinocytes displayed an induction of anagen, a proliferative phase of hair follicles, which requires activation of stem cells in the hair follicle bulge region (28). This stimulation of anagen following conditional $TERT^{ci}$ upregulation occurred with similar efficiency as wild-type TERT (28), demonstrating the ability of TERT to activate hair follicle stem cells and trigger hair growth independent of its reverse transcriptase activity. Further microarray analyses of the transcriptional profiles of mouse skin following acute perturbations in TERT levels revealed differential expression of genes involved in development/morphogenesis, signal transduction, and cytoskeleton/cell adhesion signaling pathways (28). Using pattern-matching algorithms, this TERT-regulated transcriptional program was found to overlap closely with those controlled by Wnt and Myc (28). This close resemblance of the TERT signature to Wnt- and Myc-regulated transcriptional programs suggests a potential intersection of TERT in the Wnt and Myc pathways, which are known to exert cancer-promoting effects by virtue of their fundamental roles in controlling proliferation, differentiation, and stem cell renewal. The first evidence of the direct regulation of Wnt/β-catenin signaling by telomerase was subsequently uncovered by Park and colleagues in mouse embryonic stem cells and *Xenopus laevis* embryos. In their study, TERT was reported to act as a cofactor in the β-catenin transcriptional complex through its interaction with Brg1, a SWI/SNF (SWItch/Sucrose NonFermentable)-related chromatin remodeling factor (29). Overexpression of TERT or $TERT^{ci}$ resulted in the activation of Wnt-dependent reporters *in vitro* and *in vivo*, whereas chromatin immunoprecipitation assays revealed TERT occupancy at promoters of Wnt target genes such as *cyclin D1* and *c-Myc* in mouse intestine (29). Intriguingly, TERT or $TERT^{ci}$ overexpression in *Xenopus* embryos, in conjunction with β-catenin, promoted anterior–posterior axis duplication (29). In contrast, mice deficient in TERT displayed homeotic transformations in their vertebrae (29). These observations reveal a noncanonical function for TERT as a key regulatory component of the Wnt/β-catenin transcriptional complex, exerting a profound effect in developmental processes controlled by Wnt signaling. This synergistic role of telomerase on the transcriptional activity of Wnt/β-catenin is independent of its catalytic function at telomeres. In a more recent study by Okamoto and colleagues, TERT was also illustrated to form a complex with Brg1, together with nucleostemin (NS), a nucleolar GTP-binding protein and/or its family member GNL3L (30). This NS/GNL3L–TERT–Brg1 complex was found to be essential for maintenance of the tumor initiating cell phenotype in human cancer cells, thereby extending support for the regulatory role of TERT in the Wnt/β-catenin pathway through Brg1 interaction (30).

Although two recent studies from the Blackburn and Greider laboratories have reported a lack of physical interaction between TERT and Brg1 or β-catenin in Hela and breast cancer cell lines (31) as well as the absence of developmental defects

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associated with defective Wnt signaling in TERT-deficient mice, respectively (32), the apparent discrepancies in effects of TERT may be attributed to the different cell type, experimental setup, mouse strain background, or laboratory environment used in the three studies. As such, the role of telomerase on Wnt signaling should not be dismissed completely but rather, be viewed as one that is context dependent and especially relevant in cancers in which multiple signaling pathways such as NF-κB and Wnt are perturbed concurrently. In addition, the finding that Wnt/β-catenin signaling can in turn regulate telomerase transcriptionally in embryonic stem cells and human cancer cells (14, 15) further fuels the notion of a feed-forward regulatory cycle that sustains telomerase activity and amplifies oncogenic signaling mechanisms concurrently.

This feed-forward signaling loop that is proposed to exist between TERT and NF-κB signaling as well as TERT and Wnt/β-catenin signaling in cancers is a likely mechanism for the concomitant activation of NF-κB- and Wnt/β-catenin–dependent transcription following TERT overexpression, thereby driving the prolonged expression of target genes critical for the maintenance of tumor survival and proliferation (Fig. 1). Interestingly, the observations that c-Myc, a known transcriptional target of Wnt/β-catenin signaling, regulates *TERT* expression (12) and that NF-κB modulates Wnt signaling during tumorigenesis (33) add further complexity to the cross-talk and functional cooperativity between telomerase and multiple signaling networks associated with cancer progression. Wnt/β-Catenin signaling can also activate the transcription of *TRF2*, whereas β-catenin downregulation in cancer cells aggravates telomere dysfunction (34). These data illustrate the interaction of Wnt/β-catenin signaling with telomere stabilization pathways and further corroborate existing evidence of the cross-talk between telomerase and telomere-binding proteins with cancer-promoting signaling pathways.

Telomerase-Targeted Cancer Therapies: Potential Interventions and Future Perspectives

In view of the evidence mentioned earlier linking the noncanonical functions of telomerase to cancer development and progression, targeting telomerase as an anticancer strategy seems to be an effective approach to simultaneously dampen oncogenic signaling pathways that are augmented by telomerase and disrupt the feed-forward regulatory mechanism

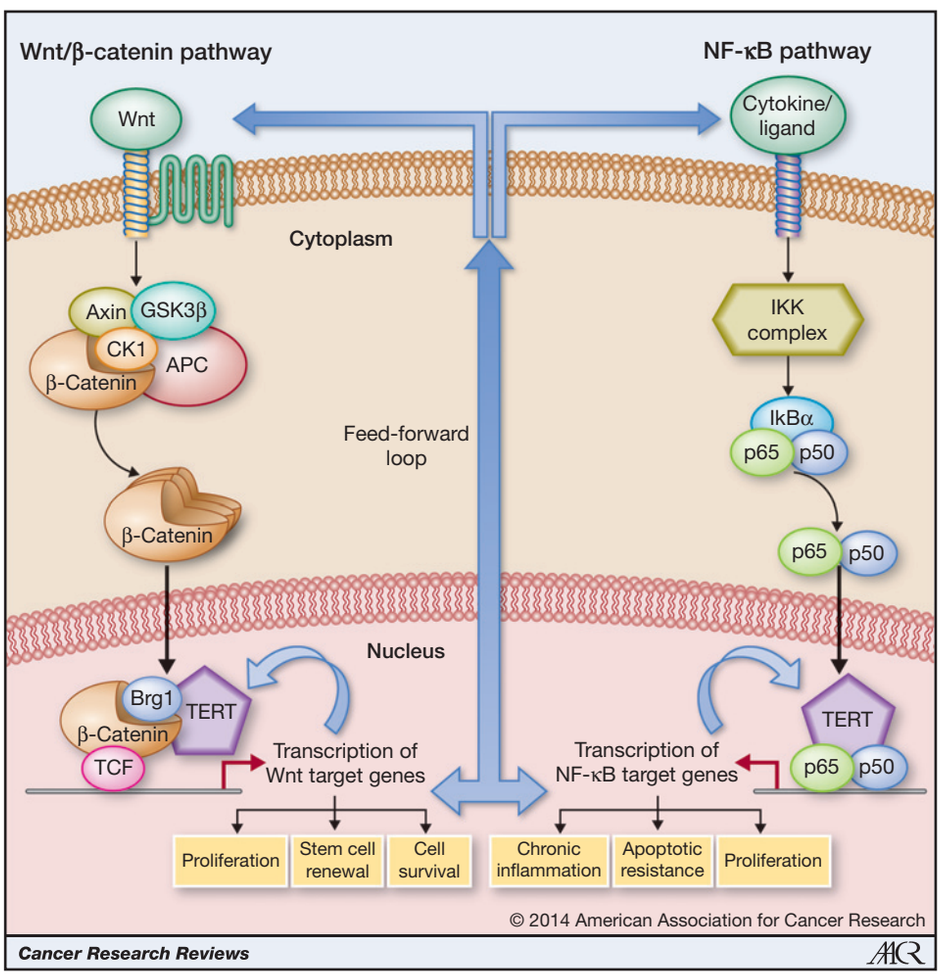

Figure 1. The proposed model of the feed-forward regulatory loop underscoring the interaction of TERT with the Wnt/β-catenin and NF-κB signaling pathways during cancer development. Reactivated TERT acts as a transcriptional modulator of Wnt/β-catenin and NF-κB signaling, resulting in the enhanced expression of Wnt and NF-κB target genes that exert cancer-promoting functions such as proliferation, resistance to apoptosis, and chronic inflammation. As Wnt/β-catenin and NF-κB are also transcriptional activators of TERT, we suggest a feed-forward pathway (illustrated by blue arrows) that sustains Wnt/β-catenin and NF-κB–dependent transcription as well as levels of telomerase in cancer cells in a simplified schematic of signaling events.

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driving chronic inflammatory/oncogenic responses and sustained telomerase activity in cancers. Furthermore, as telomerase is often upregulated in cancer cells, whereas majority of normal somatic cells have undetectable telomerase activities, telomerase-targeted cancer therapies serve to selectively eliminate tumor cells and avoid the adverse side effects. Several studies in the recent years have contributed to the rapid emergence of data depicting the multiple noncanonical functions of telomerase in cancer progression in a manner that is independent of its well-recognized role in telomere maintenance. This accumulating evidence indicates a compelling rationale for the development of therapeutic approaches that target the noncanonical roles of telomerase, instead of solely relying on conventional small-molecule inhibitors that restrict its enzymatic activity or accessibility/function at telomeres.

Taking into account the evidence that telomerase exerts its tumor-promoting roles via transcriptional modulation of the NF-κB and Wnt/β-catenin pathways, therapeutic approaches that aim to impair the interactions of TERT with NF-κB or β-catenin can be devised as a strategy to check tumor growth and survival. Herein, further studies examining the interacting domains essential for the physical association between TERT and NF-κB or β-catenin may prove useful for the treatment of cancers displaying aberrant NF-κB or Wnt/β-catenin signaling. This strategy can also be applied to other telomerase components such as Pontin and Reptin, which have also been found to interact with major oncogenic factors such as β-catenin and c-Myc (9). Meanwhile, the search for novel-interacting partners of TERT and other telomerase components that are involved in cancer-promoting pathways as well as novel epigenetic regulators essential for TERT recruitment to NF-κB or Wnt/β-catenin target genes will generate a valuable resource for the future development of drugs targeting the specific oncogenic activity of relevant telomerase components. Therapeutic approaches that block the transcriptional activation of telomerase can also be used as a way to disrupt the cross-talk between telomerase and tumor-promoting pathways. Thus, understanding how oncogenic transcription factors such as NF-κB, c-Myc, and Wnt/β-catenin bind to the TERT promoter and activate its expression will be useful for the design of drugs or oligos that inhibit the recruitment of transcriptional activators at TERT promoter. This approach can be used in combination with the current telomerase-based immunotherapy strategies that aim to elicit an immune response in cytotoxic $CD8^+$ T cells against tumor cells displaying TERT peptides on their surface (9). Hence, inhibiting the expression of telomerase as well as targeting telomerase-positive cancer cells serves to interrupt the feed-forward regulatory loop that sustains cancer-promoting signaling pathways. The discovery of the noncanonical roles of telomerase in cancer creates a promising avenue for the development of effective anticancer therapies that specifically target the oncogenic abilities of telomerase. Targeting the noncanonical functions of telomerase, in combination with drugs that inhibit the catalytic activity of telomerase, as a therapeutic approach can prevent the persistent activation of major oncogenic signaling pathways while concurrently inducing senescence in proliferating tumor cells as a result of critical telomere shortening. This strategy can be an efficient way to trigger the regression of telomerase-positive tumors with minimal cytotoxicity to normal tissues and may prove superior to the current anticancer treatment options. Thus, future studies that explore the noncanonical functions of telomerase components, mechanisms of action as well as novel coactivators, and interacting partners are highly warranted for the rational design of effective therapeutic interventions against telomerase-active cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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