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Survival of APC-mutant colorectal cancer cells requires interaction between tankyrase and a thiol peroxidase peroxiredoxin II

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Overexpression of mammalian 2-Cys peroxiredoxin (Prx) enzymes is observed in most cancer tissues. Nevertheless, their specific role in colorectal cancer progression has yet to be fully elucidated. Here, a novel molecular mechanism by which PrxII/TNKS interaction mediates survival of APC-mutant CRC cells was explored. In mice with inactivating *APC* mutation, a model of spontaneous intestinal tumorigenesis, deletion of *PrxII* reduces intestinal adenomatous polyposis and thereby increases survival. In *APC* mutation-derived human CRC cells, PrxII depletion hinders the PARP-dependent Axin1 degradation through TNKS inactivation. H_2O_2-sensitive Cys residues in zinc-binding domain of TNKS1 was found to be crucial for its PARsylation activity. Mechanistically, direct binding of PrxII to ARC4/5 domains of TNKS confers a vital redox protection against TNKS oxidative inactivation. As a proof-of-concept experiment, a chemical compound targeting PrxII inhibits the growth of tumors xenografted with *APC*-mutation-positive CRC cells. Collectively, the results provide evidence revealing a novel redox mechanism for regulating TNKS activity in such that physical interaction between PrxII and TNKS promotes survival of APC-mutant colorectal cancer cells by PrxII-dependent antioxidant shielding.

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Abbreviations: Prx, Peroxiredoxin; CRC, Colorectal cancer; APC, Adenomatous polyposis coli; FAP, familial adenomatous polyposis; Axin1, Axis inhibition protein 1; CK1, Casein kinase 1; GSK, Glycogen synthase kinase; ROS, Reactive oxygen species; TNKS, Tankyrase; ARC, Ankyrin repeat cluster; PARP; poly (ADP-ribose) polymerase

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In most cancer tissues, overexpression of mammalian 2-Cys peroxiredoxin (Prx) enzymes is observed. Colorectal cancer (CRC) with the presence of inactivating mutations in the adenomatous polyposis coli (*APC*) suppressor gene has been widely studied. APC is a key scaffold protein in the β-catenin destruction complex, composed together with axis inhibition protein 1 (Axin1), β-catenin, casein kinase 1 (CK1) and glycogen synthase kinase (GSK)-3β, for efficient β-catenin degradation. Up to date, numerous studies have indicated that initiation of intestinal tumorigenesis by *APC* mutations is promoted by the acquired or inherited mutation in the DNA glycosylase enzymes essential for base excision repair of oxidative DNA damage, suggesting the involvement of elevated level of reactive oxygen species (ROS) in driving the *APC* mutation-driven intestinal tumorigenesis. Mammalian 2-Cys Prx enzymes are actually the most efficient peroxidases that catalyze the reduction of H_2O_2 to water in

the presence of NADPH by coupling with the thioredoxin/thioredoxin reductase system. It has been well established that 2-Cys Prx enzymes have the multifaceted roles in cellular ROS detoxification and signal transduction. However, the molecular mechanism regulating tankyrase (TNKS) activity in CRC remains largely unknown. This study primarily focused on elucidating the molecular mechanism between TNKS and a thiol peroxidase named PrxII necessary for the survival of CRC cells.

Somatic mutations on oncogenes and tumor suppressors cause intrinsic oxidative stress in cancer cells by amplifying ROS production. ROS has been recognized to have a double-edged function, where a moderate and transient induction of cellular ROS level is undoubtedly required for hyper-proliferation of cancer cells due to a second messenger role in growth factor signaling. On the contrary, excess ROS level is mutagenic and cytotoxic due to the oxidative damages on macromolecules. Kang *et al* shows that loss of PrxII elevated level of ROS and unexpectedly inhibited intestinal tumorigenesis induced by *APC* mutation, which further confirms that H_2O_2 level regulated by PrxII is critical for the survival of tumorigenic epithelial cells in mouse intestinal adenomas. Stringent knockdown of peroxidase activity of PrxII drastically reduced the levels of total and active (unphosphorylated) β-catenin via the canonical destruction complex only in CRC cells with *APC* mutation. When *APC* is mutated, functional Axin1 becomes important for regulation of β-catenin levels. Level of Axin1 is known to be regulated by the poly (ADP-ribose) polymerization (PARsylation) and its sequential ubiquitination. Depletion of PrxII blocked PARsylation/ubiquitination of Axin1 without affecting total ubiquitination and caused the augmentation of functional Axin1-associated destruction complexes to degrade β-catenin, hence reversing the oncogenic phenotype of *APC* mutation. Together, these results suggested that H_2O_2 regulated by PrxII intimidates the tumorigenic epithelial cells by targeting TNKS in growing intestinal tumors and is not necessary for classical Wnt/ β-catenin signaling as *APC* mutations induces the Wnt-independent accumulation of transcriptionally active β-catenin.

TNKS, a sole PARsylating enzyme for regulating the level of Axin proteins, specifically interact with the glycine residue at position 116 of PrxII through its ankyrin repeat cluster (ARC) 4/5 domains. Endogenous Axin1 proteins are tightly controlled by TNKS-dependent degradation via PARsylation and subsequent ubiquitination in CRC cells. It was shown for the first time that absence of PrxII reduced oncogenic β-catenin in the adenomatous polyps as well as the APC-mutant CRC cells due to the Axin1-dependent β-catenin degradation. It was also intriguing result that H_2O_2-dependent inactivation of TNKS1 PARP activity was happened by the loss of zinc ions from the PARP domains. Taken together, the results reveal a novel redox mechanism by which a zinc-binding motif essential for the PARP activity of TNKS is vulnerable to oxidation and requires the PrxII-dependent antioxidant shielding effect (**Figure 1**). Presently, there have been no isoform-specific chemical compounds that enable the inhibition of peroxidase activity of human PrxII. Conoidin A, a cell-permeable compound that covalently binds to parasite PrxII, was tested and shown to sufficiently inhibit PrxI and PrxII activities in a slightly different mechanism. Promisingly, conoidin A inhibited colony-forming growth in APC-mutant CRC cells, but not APC-competent CRC cells. Conoidin A treatment against tumor xenografts derived from APC-mutant CRC cells significantly retarded the tumor growth. These results

imply that PrxII inhibitor can be a new therapeutic weapon for combating with CRC with *APC* mutations.

In conclusion, the study provides the intrinsic mechanistic evidence for a tumor-promoting role specific to PrxII, but not PrxI, beyond its canonical antioxidant role. Specifically, PrxII protects TNKS activity by direct binding to TNKS via ARC4/5 domains in cytosol and consequently reserves deregulated β-catenin pathway in APC-mutant CRC cells. Hence, targeting of PrxII may exert specific and broad therapeutic potentials for treating familial adenomatous polyposis (FAP) as well as *APC*-mutation-harboring CRCs.

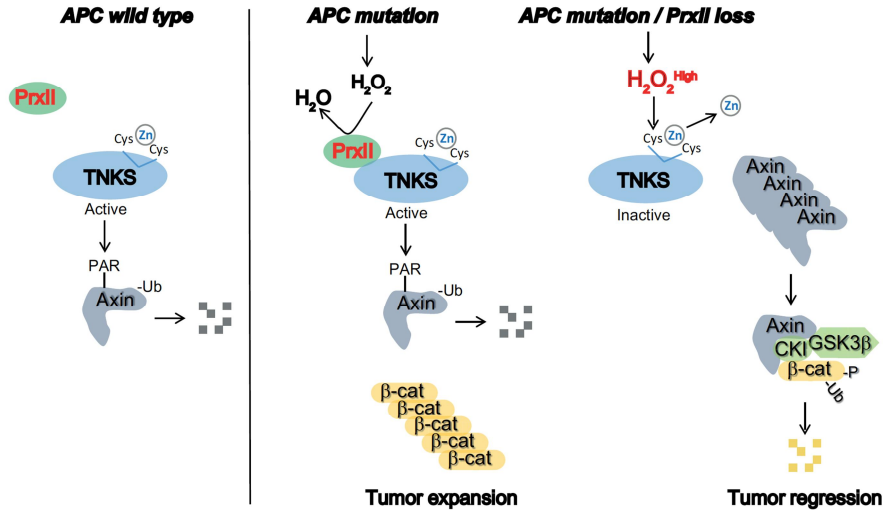

Figure 1. Scheme for mechanistic model by which PrxII promotes survival of CRC cells.
The level of intracellular H_2O_2 elevates in the presence of *APC* mutation, which in turn recruits and induces PrxII binding to TNKS. PrxII eliminates vicinal H_2O_2 around TNKS to shield the Cys residues in zinc-binding motif upon oxidative stress, which renders the liberation of zinc ions from the PARP domains. As a result, PrxII binding to TNKS confers a protective function against the oxidative inactivation of TNKS PARP activity and consequently degrades Axin1 that promotes the accumulation of β-catenin hence enforcing the proliferation and survival of CRC cells. Conversely, the loss or inhibition of PrxII in concert with *APC* mutation prevents the reduction reactions of the intracellular H_2O_2 level, which then triggers the Cys oxidation and leads to the release of zinc ions and inactivation of TNKS. Soon after, Axin1 proteins are accumulated and form the canonical β-catenin destruction complex. Destruction of β-catenins results in apoptotic cell death and regression of CRC tumor.

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