The Biology Behind

Ribonucleotide Reductase Subunit One as Gene Therapy Target


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RNR \(^2\) catalyzes the reaction in which 2'-deoxyribonucleotides are generated from the corresponding ribonucleotide 5'-diphosphates. This is the rate-limiting step in the production of 2'-deoxyribonucleoside 5'-triphosphates required for DNA replication. RNR consists of two protein subunits, R1 and R2.\(^3\) The R1 subunit is a \(M_r\) 160 KD homodimer that contains the catalytic site, two allosteric effector-binding sites, and redox active disulfides that participate in the reduction of substrates (1, 2). The R2 subunit is a \(M_r\) 78 KD homodimer that contains a nonheme iron that participates in catalysis by forming an unusual free radical on the aromatic ring of a tyrosine residue. Both the R1 and the R2 subunits are required to form the active site of the enzyme, with its genes located on separate chromosomes and the corresponding mRNAs differentially expressed throughout the cell cycle. The level of R1 protein remains relatively stable throughout the cell cycle, whereas R2 expression is cell cycle dependent, with highest expression concurrent with DNA replication. The RNR enzymatic activity is also regulated by allosteric control mechanisms involving positive and negative effectors (2, 3).

Although best characterized as the large subunit of the RNR complex, R1 may also be the large subunit component of the complex responsible for the generation of 2'-deoxyribonucleoside 5'-triphosphate for DNA repair. p53R2, a recently identified p53-regulated R2 parologue, is the putative small subunit of the ribonucleotide reductase complex involved in DNA repair (4–6). R1 has been shown to be up-regulated in response to DNA damage, consistent with a role in DNA repair (4–6). R1 has been shown to be up-regulated in response to DNA damage, consistent with a role in DNA repair (4–6).

The R1 gene has been mapped to chromosome 11p15.5 (20). Interestingly, the centromeric part of 11p15.5 contains a region of frequent LOH in many solid malignancies including lung, breast, ovarian, and stomach cancers (21–27). LOH resulting from an allelic loss of a polymorphic locus is often useful in the identification of tumor suppressor genes. The frequency of LOH within this region is also correlated with metastatic tumor spread (28, 29). Genetic complementation studies using chromosome 11 and fragments containing segment 11p15.5 resulted in reduced tumorigenesis in a syngeneic mouse model and growth inhibition of a number of tumor cell lines \textit{in vivo} (30–35). Results suggest that R1, encoded within the region of frequent allele loss, may account for at least part of the observed tumor-suppressing activity in the 11p15.5 region.

The article by Cao \textit{et al.} (36) investigates the potential of R1 gene therapy for human cancer using a recombinant adenovirus encoding the human R1 gene (\textit{rAd5-R1}). A recombinant adenoviral vector, \textit{rAd5-R1}, was produced by site-specific recombination and \textit{in vitro} expression studies showed adenovirus-mediated overexpression of R1 compared with vehicle and \textit{rAd5-LacZ} controls. Transduction of \textit{rAd5-R1} into human colon adenocarcinoma cells (Colo320 HRS) induced antiproliferative effects in a dose- and time-dependent manner. It was observed that the proliferation of normal cells is not affected by R1 overexpression, which is consistent with R1 acting as a tumor-specific growth suppressor. These inhibitory effects were demonstrated in animal models. The \textit{rAd5-R1} not only suppress tumorigenesis after \textit{ex vivo} treatment of Colo320 HRS cells, but it also suppressed the growth of Colo320 HRS xenografts when injected intratumorally. Both experiments revealed a significant effect on tumor growth compared with the effect after treatment with a lacZ-expressing recombinant virus, which demonstrated...
sequence specificity. These results suggest that the inhibition of tumor growth in vitro and in vivo is mediated by overexpression of R1. This is the first published report describing antitumor effects of adenovirus-mediated overexpression of the R1 component of human RNR, which extends previous observations that suggested the R1 component of RNR is a putative tumor suppressor (12).

Development of colon cancer often involves multiple genetic alterations including a high incidence of LOH and activation of a number of oncogenic pathways (37). A large number of colon cancer cell lines have been classified by 31 known genetic alterations (37). All of the cell lines examined in the Cao et al. publication (36) are included in the classification. However, there was no clear correlation observed between the in vitro
sensitivity to R1 overexpression and LOH at a number of loci. Also, chromosome 11 was not included in the analysis. No extensive analysis was done showing which colon cancer cell lines have LOH at chromosome 11p15.5. The possibility that sensitivity to R1 overexpression is related to LOH at 11p15.5 is currently being investigated. Furthermore, there was no correlation between p53 status and sensitivity to R1 overexpression. Tumor cell lines containing both wild-type and mutant p53 were sensitive, which suggests that the activity is not directly linked to p533R2 activity. Of 31 genetic alterations known to occur in colon cancer, the only correlation that we could find was with ras mutations. All of the cell lines tested that were sensitive to R1 overexpression had a mutation in the ras gene. The insensitive cell lines were wild type for ras. This observation, together with previous findings on ras/R2 synergism (11), suggests that there may be some convergence of RNR components and the major cell cycle regulatory pathway, which is also supported by earlier studies. This is a hypothesis that will be actively studied in the future.

Some tumor suppressor genes, such as p53, p16, and pRB, have been investigated for cancer gene therapy. In particular, the p53 tumor suppressor gene has displayed antitumor efficacy against human cancers including colorectal, prostate, breast, cervical, ovarian, and skin cancers (38–43). Cao et al. (36) have used the tumor suppressing activity of the R1 component of human RNR. Allelic loss of R1 is frequently observed in the LOH11A region on chromosome segment 11p15.5 in a variety of tumors. This, together with previous data (44), genetic complementation studies (31–35), and clinical correlative studies (22–25, 27, 28) support the hypothesis that a gene capable of tumor suppressing activity is located in the LOH11A region and that the most likely candidate is the R1 gene.

R1 appears to be quite different from “classical tumor suppressor genes.” For example, loss-of-function mutations in p53 are usually recessive, and cancer occurs only when both copies are defective. In contrast, complete loss of R1 is deleterious to cell survival. This is because RNR activity is essential for de novo deoxyribonucleotide synthesis and maintenance of dNTP pools during DNA replication. The unusual finding that a screen of 117 lung cancer cell lines did not identify any homozygous deletions in the LOH11A region (45) support the hypothesis that at least one functional allele of the R1 gene is required for cell viability, whereas normal or elevated levels of R1 expression result in a suppression of phenotypic characteristics of malignant cells. The malignant phenotype associated with the allelic loss in the LOH11A region likely results from a recessive or null mutation in one allele of R1 rather than a homozygous recessive mutation, categorizing R1 as a novel tumor suppressor. It would appear that Knudson’s “two-mutation” criterion (46) does not sufficiently explain tumor suppressors such as R1. This type of “haplo-insufficiency” for tumor suppression has also been observed for p27^Kip1, a candidate tumor suppressor protein that inhibits cyclin-dependent kinases and blocks cell proliferation (47–49).

Different mechanisms control R1 and R2 gene expression and enzyme activity during cell proliferation (50). Transcription of both of the R1 and R2 genes occurs during S phase, but the R2 protein is primarily expressed in S phase and slowly accumulates up to late mitosis when it is rapidly degraded (51). The level of R1 protein is constant during the cell cycle in proliferating cells because of its long half-life (52). Therefore, the RNR activity is controlled by the synthesis and degradation of the R2 protein during the cell cycle. In an in vitro study, overexpression of R1 in human oropharyngeal carcinoma KB cells had a negligible effect on RNR enzymatic activity and did not alter the R2 expression, although R1-overexpressing cells demonstrated decreased invasive potential compared with control cells (13, 16). In contrast, overexpression of R2 in KB cells resulted in a significant increase in the enzymatic activity of RNR and invasive potential (16). When R2 expression in rAd5-R1-transduced cells was examined, there was no significant change in the R2 protein expression. Given that the R2 protein is limited in cells, R1 protein overexpression alone would not be expected to increase RNR activity. This suggests that R1-mediated tumor suppressing activity is not a direct consequence of changes in RNR activity.

The mechanism underlying the tumor-suppressing activity of R1 remains uncertain. There is the possibility that R1 exerts its tumor-suppressing activity simply through deregulation or competitive depletion of dNTP pools by increasing levels of ATP and dNTP binding to the R1 subunit. However, this is unlikely considering that other tumor cell lines including human metastatic melanoma (A2058) cells were not affected by rAd5-R1 transduction, even though the R1 protein was highly overexpressed (data not shown). Furthermore, in a previous study, tumorigenesis of mutant ras-, p53-, and c-myc-transformed mouse fibroblast 10T½ cell line, infected with R1 retroviral construct (RMP/mR1), was not affected in soft agar assays (11). Although there is no direct evidence, it is attractive to speculate that excess R1 modulates growth-signaling pathways and thus affects cell proliferation, differentiation, and apoptosis. This is supported by the observation that deregulated R2 expression activates the mitogen-activated protein kinase pathway and alters cell proliferation, differentiation, and apoptosis (44). The observed correlation between the inhibition of colon tumor cell growth in vitro and ras mutation would support this hypothesis. Extensive studies are still needed to determine whether this correlation reflects a direct causal relationship.

Given that R1 may be the large subunit for the p53R2 ribonucleotide reductase complex, R1 expression may directly affect DNA repair mechanisms via interaction with p53R2. As with RNR, this pathway has the potential to alter tumorigenesis and growth signaling pathways. The lack of correlation between the p53 state and inhibition of colon cancer cell proliferation in vitro would argue against this hypothesis but, without additional experimentation this pathway, cannot be ruled out. A clear mechanism by which R2 (oncogenic stimulator) and R1 (oncogenic suppressor) are involved in tumorigenesis has not been elucidated, but, taken together, these studies support the possibility that RNR components play more than a passive dNTP synthetic role in cell cycle progression and that their expression alters the signaling pathways that govern cell proliferation and, potentially, metastasis. Aside from the therapeutic potential of modulating RNR components is the intriguing concept that R1/R2 (and potentially p53R2) stoichiometry can regulate cell proliferation and tumorigenic potential. This is an open and compelling hypothesis that brings RNR back to the forefront of scientific interest.
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References


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