Background: To determined the safety and efficacy of recombinant adeno-viral human p53 gene (rAd-p53) combined with FOLFOX4 in treatment of advanced colorectal cancer. Methods: Twenty-two patients with advanced colorectal cancer were treated using rAd-p53 combined with FOLFOX4 from July 2008 and April 2010. Intratumoral injection of rAd-p53 at a dose of 1-4×10^{12} viral particles (VP) was given to all the patients once per week for two week. If tumor spreading into abdominal cavity, 3×10^{12} VPs diluted in 500 mL of saline solution were injected intraperitoneally after removing suitable mount of peritoneal fluid twice in 2 weeks. If having lung or liver metastasis, 2×10^{12} VPs were given intravenously twice in 2 weeks. Three days after starting gene therapy, one cycle of systemic chemotherapy (FOLFOX4 regimen) was given to all these patients. Results: All patients were alive during follow-up period (68~766 days). One patient was assessed as complete response, 5 as partial response, 2 as stable disease, and 5 patients as progressive disease. After the combined treatment, a radical surgery was successfully performed in 12 cases. KPS score was evaluated after the end of treatment as 90~100 in 15 cases (68.2%), 90~70 in 5 cases (22.7%), and 70~50 in 2 cases (9.1%), compared to that of 90~70 in 13 cases (59.1%), 50~30 in 4 cases (18.2%), and 30~10 in 1 case (4.5%) before starting treatment. Self-limiting fever was reported in 10 cases (45.5%), and nausea and diarrhea in 1 case (4.5%) after rAd-p53 administration. Conclusions: RAd-p53 combined with FOLFOX4 is a safe and effective treatment for advanced colorectal cancer.