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Short Communication

APC10.1 cells as a model for assessing the efficacy of potential chemopreventive agents in the Apc^{Min} mouse model *in vivo*

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ABSTRACT

Apc^{Min} mice are widely used for mechanism and efficacy studies associated with the development of chemopreventive agents. APC10.1 cells have been derived from Apc^{Min} mouse adenomas and retain the heterozygous Apc genotype. We tested the hypothesis that this cell type may provide an *in vitro* model to predict chemopreventive activity of agents in the Apc^{Min} mouse *in vivo*.

The growth inhibitory properties of 14 putative colorectal cancer chemopreventive agents, tricin, apigenin, 3′,4′,5′,5,7-pentamethoxyflavone, resveratrol, curcumin, 3,4-methylenedioxy-3′,4′,5′-trimethoxychalcone (DMU135), 3,4,5,4′-tetramethoxystilbene (DMU212), celecoxib, aspirin, piroxicam, all-*trans*-retinoic acid, difluoromethylornithine (DFMO), quercetin and cyanidin-3-glucoside, were studied in this cell line, and the IC_{50} values were calculated. The IC_{50} values were plotted against previously published data of reduction of adenoma numbers caused by these agents in Apc^{Min} mice. The correlation co-efficient was 0.678 ($p < 0.01$), suggesting that there was a tentative correlation between the ability to inhibit the growth of APC10.1 cells and the ability to delay adenoma development *in vivo*. If this relationship is supported by using further agents, APC10.1 cells may serve in the future as an initial screen to prioritise compounds for assessing chemopreventive efficacy in Apc^{Min} mice *in vivo*. Such a screen could reduce the number of animals required to find active agents, help reduce costs and increase throughput.

1. Introduction

Rodent models of colorectal carcinogenesis are useful tools for the development of novel cancer chemopreventive agents, as they allow assessment of the effect of intervention on incidence, multiplicity or burden of tumours. The Apc^{Min} mouse, has a chemically induced mutation of the *Apc* gene at codon 850, which is also mutated in the familial adenomatous polyposis (FAP) syndrome and most colorectal adenocarcinomas in humans.[1] Apc^{Min} mice develop tumours predominantly in the small intestinal tract, whereas the human condition is characterised by adenomas mainly in the colon. A retrospective analysis of chemopreventive activity of cyclooxygenase (COX) inhibitors as colorectal cancer chemopreventive agents

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in rodents and humans suggests moderate consistency between species,[2] supporting the relevance of rodents for cancer chemoprevention research. The *Apc*^{Min} mouse model predicted the adenoma-regressing activity of the NSAID sulindac and the COX-2 inhibitor celecoxib[3,4] in familial adenomatous polyposis coli patients.[5,6] Testing putative chemopreventive drug candidates in rodents is expensive and time-consuming, with *Apc*^{Min} studies usually lasting up to 18 weeks. Therefore there is a need for a more rapid screen that will allow drug discovery to be more efficient. It is therefore proposed that tests *in vitro* which would allow prioritisation of agents for study in rodents *in vivo* are most desirable. The APC10.1 is a new intestinal cell line derived from *Apc*^{Min} mice that retains both the heterozygous Apc genotype and a non-activated Wnt signalling pathway, and displays an early neoplastic phenotype.[7] It has been proposed that this cell line could constitute a novel *in vitro* model that, coupled with *in vivo* studies performed in *Apc*^{Min} mice, could contribute to a better understanding of the human condition FAP and to the discovery of new therapeutic approaches.[7] Here we test the hypothesis that APC10.1 cells serve as predictors of activity of potential chemopreventive agents in *Apc*^{Min} mice *in vivo*. We studied the effect of 14 putative colorectal cancer chemopreventive agents on APC10.1 cell proliferation and assessed the correlation between the IC_{50} values and the ability of these agents to compromise adenoma development *in vivo* in *Apc*^{Min} mice.

2. Materials and methods

2.1. Materials

Agents were purchased from Sigma (Poole, UK) unless otherwise stated. Resveratrol was purchased from Changchun Kingherb International (Changchun, China), 3,4-methylenedioxy-3′,4′,5′-trimethoxychalcone (DMU135) and 3,4,5,4′-tetramethoxystilbene (DMU212) were kind gifts from Prof Gerry Potter, De Montfort University (Leicester, UK), celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzene-sulphonamide) was provided by Pfizer Ltd. (Sandwich, UK) and cyanidin-3-glucoside was a kind gift from Indena SpA (Milan, Italy). Tricin was custom-synthesised for the US NCI Division of Cancer Prevention by Syncom (Groningen, The Netherlands). Apigenin and pentamethoxyflavone were purchased from Apin Chemicals Ltd. (Abingdon, UK).

2.2. Cell culture

APC10.1 cells were kindly provided by Dr Carla De Giovanni from the Cancer Research Section (University of Bologna, Italy). APC10.1 cells were cultured in Dulbecco's-modified Eagle medium supplemented with 20% foetal bovine serum (Gibco, Paisley, UK) and harvested by a 15–30-min treatment with trypsin-EDTA solution (Gibco, Paisley, UK). The cells were used up to passage number 20. The cells were seeded (2×10^3) onto 24-well plates and allowed to adhere overnight, then agents dissolved in DMSO were added and the cells were incubated for periods of up to 144 h to furnish a final DMSO concentration of <0.1%. The cells were washed with phosphate-buffered saline (PBS), harvested by trypsinisation and resuspended in cell culture media (1 ml). Aliquots (1 ml) were diluted 10-fold (Isoton II buffer) and 500 μl was analysed using a Z2 Coulter Particle Count and Size Analyser (Beckman Coulter, UK). Incubations were conducted in triplicate. The IC_{50} values were calculated from the plot of cell number as percentage of DMSO control *versus* agent concentration at 144 h, at which time the cells were still in linear growth phase. The values are the mean ± SD of the three separate experiments.

2.3. *Apc*^{Min} mouse studies

The data on the effect of the agents on adenoma number were obtained either in-house or from D. Corpet's chemoprevention database http://www.inra.fr/reseau-nacre/sci-memb/corpet/indexan.html (Ecole Nationale Vétérinaire de Toulouse, France). All the data used in this study were from animals that had been dosed with agents admixed with their normal diet. The dietary concentrations were as follows: tricin 2000 ppm,[8] apigenin 2000 ppm,[9] 3′,4′,5′,5,7-pentamethoxyflavone 2000 ppm,[10] resveratrol 2000 ppm,[11] curcumin 1000–2000 ppm,[12–14] DMU135 2000 ppm,[15] DMU212 2000 ppm,[11] celecoxib 300–1500 ppm,[4,16] aspirin 200–500 ppm,[17–19] piroxicam 25–200 ppm,[20–22] all-*trans*-retinoic acid 10 ppm,[23] difluoromethylornithine (DFMO) 10,000–20,000 ppm,[20,24] quercetin 20,000 ppm[13] and cyanidin-3-glucoside 3000 ppm.[25] For agents for which multiple investigations have been published adenoma reduction was meaned. To avoid any bias, studies were only meaned where similar concentrations were used and also the same route of administration.

2.4. Statistical analysis

The significance of the correlation between the IC_{50} value in cells and the adenoma number was evaluated by a two-tailed Pearson's correlation coefficient using the Statistical Package for the Social Sciences (SPSS) version 16 programme (Windows XP).

3. Results

The effect of the following agents on the growth of APC10.1 cells was studied: tricin, apigenin, 3′,4′,5′,5,7-pentamethoxyflavone, resveratrol, curcumin, 3,4-methylenedioxy-3′,4′,5′-trimethoxychalcone (DMU135), 3,4,5,4′-tetramethoxystilbene (DMU212), celecoxib, aspirin, piroxicam, all-*trans*-retinoic acid, difluoromethylornithine, quercetin and cyanidin-3-glucoside. The IC_{50} values were calculated on day 6 (Table 1). The effect of exposure of *Apc*^{Min} mice to these agents on the adenoma number was derived from the studies conducted previously in our laboratory or reported in the Corpet database. Table 1 shows the adenoma number observed in mice exposed to these agents as percentage of control mice which did not receive the intervention. An XY scatterplot of the IC_{50} value *versus* the adenoma number was plotted, with a line of best fit, and the correlation co-efficient was calculated as 0.678 with $p < 0.01$ (Fig. 1).

4. Discussion

The aim of this study was to delineate any association between the IC_{50} values for inhibition of the growth of