Preclinical *in vitro* models from genetically engineered mice for breast and colon cancer (Review)

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Abstract. Genetically engineered mice with targeted alterations in clinically relevant oncogenes, tumor suppressor genes or DNA mismatch repair genes provide unique predictive animal models for human carcinogenesis, and cancer prevention/ therapy. However, some of the genetically engineered mouse models lack target organ specificity for colon carcinogenesis. We have established, characterized and validated stable epithelial cell lines from 'normal' and 'genetically' predisposed target organs that offer innovative and mechanistic approaches, complementing *in vivo* studies on existing animal models for clinical breast and colon cancer. Epithelial cell lines with up-regulated Ras or myc oncogene, mutated Apc tumor suppressor gene and Mlh_1 DNA mismatch repair gene provide facile experimental systems for organ site carcinogenesis and cancer prevention. Altered expression of cancer specific biomarkers and their modulation by several synthetic pharmacological agents such as retinoids, selective estrogen receptor modulators, non-steroidal anti-inflammatory drugs and specific enzyme inhibitors have been reported from our laboratory. Oncogene expressing MMEC-Ras and MMEC-myc mammary epithelial cells, Apc mutant 850^{Min} COL and 1638N COL, and DNA mismatch repair/Apc mutant Mlh_1/1638N COL colon epithelial cells exhibit aberrant cell cycle progression, down-regulated apoptosis and enhanced carcinogenic risk *in vitro* and tumor formation *in vivo*. We have reported that relative to the parental 'normal' non-neoplastic cells, genetically 'altered' pre-neoplastic cells exhibit enhanced sensitivity for growth arrest by multiple mechanistically distinct pharmacological agents. Comparative experiments on isogenic 'normal' and genetically 'altered' target cell lines facilitate cancer selective efficacy and identification of susceptible mechanistic pathways. Treatment of these genetically 'altered' pre-neoplastic cells with low dose combi-nation of mechanistically distinct pharmacological agents as well as naturally occurring phytochemicals induce cytostatic growth arrest, alter cell cycle progression and reduce carcinogenic risk. The availability of validated technology for model development, and for mechanism based biomarker assays now establishes a novel platform to rapidly test carcinogenicity and preventive/therapeutic efficacy of novel pharmacological agents as well as naturally occurring phytochemicals. Thus, these data permit rational prioritization of efficacious lead compounds for preclinical testing and future clinical trials for prevention/ therapy of breast and colon cancer.

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1. Introduction

Breast and colon cancer together represent prevalent cause of mortality in the US population. Recent estimates by the American Cancer Society have projected 192,370 new cases of breast cancer and 106,100 new cases of colon cancer in 2009 (1). This survey has also projected 40,170 breast cancer related deaths and 49,920 colon cancer related deaths. The life time risk for breast and colon cancer is thus estimated to be 1 in 8 and 1 in 19, respectively. These actuarial data emphasize a persistent need for identifying specific and sensitive biomarkers for carcinogenic risk, and also for safe and effective interventional modalities.

Several elegant observations have provided scientifically robust support to the concept that gain of function mutations in oncogenes and loss of function mutations in the tumor suppressor genes represent primary predisposing genetic defects in breast and colon cancer (2-8). In accord, several genetically engineered mouse models have been developed and characterized to represent well established predictive pre-clinical animal models for clinical breast and colon cancer (5-8).

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Table I. Biomarkers for aberrant proliferation and carcinogenic risk in oncogene transfected mammary epithelial cells.

Biomarker	Cell line		
	MMEC	MMEC-Ras	MMEC-Myc
Population doubling (h)[a]	24.0	16.4	18.3
$S+G_2/M$: $subG_0$[b]	1.8 ± 0.5	9.1 ± 0.3	9.8 ± 0.6
Anchorage-independent colony[c]	0.5 ± 0.2	27.2 ± 4.0	28.0 ± 2.0
Tumorigenicity[d]	0/10	9/10	10/10
Tumor latency	24 weeks	3-5 weeks	3-5 weeks

[a]Determined at the exponential growth phase, [b]determined by flow cytometry, [c]average number of colonies at day 14 post-seeding, [d]determined by mammary fat pad transplantation.

Conventional treatment options for breast and colon cancer continue to be chemo-endocrine therapy for breast and chemo-therapy for colon. However, conventional long-term therapeutic interventional modalities are frequently associated with acquired tumor resistance and adverse systemic toxicity, compromising patient compliance (9-14).

Genetically engineered mouse models have also been extensively used as mechanism based screens to identify novel preventive/therapeutic interventional modalities (15,16). However, the genetically engineered mouse models for familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC) syndromes that express germ line mutations in clinically relevant tumor suppressor gene and/or DNA mismatch repair genes exhibit carcinogenesis predomi-nantly in the small intestine, rather than in the colon (4,6-8), while clinical FAP and HNPCC syndromes represent high risk lesions for colon cancer (3,4). Thus, these *in vivo* approaches are frequently associated with conceptual and technical limita-tions related to clinical translatability of the generated data.

Cell culture models developed from target tissue for organ site cancer that express clinically relevant genetic defects and exhibit quantifiable risk for carcinogenesis, thus represent an alternative approach that complements existing animal models for rapid mechanism based prioritization of efficacious preventive/therapeutic agents.

In the progressive pathogenesis of organ site cancer of breast and colon, intra-epithelial neoplasia such as ductal carcinoma *in situ* (DCIS) of the breast and adenoma of the colon have been considered as definitive pre-invasive lesions at risk for invasive cancer (2-4,17).

At the molecular level, DCIS are characterized by the presence or absence of hormone receptors and over expression/mutation of select genes such as Ras, myc, HER-2 and p53, and therefore, exhibit variable response to chemo-endocrine therapy (2,17,18). The colonic adenomas are characterized by germ line or somatic mutations in tumor suppressor adeno-matous polyposis coli (APC) gene or in select DNA mismatch repair genes that are associated with high risk familial adeno-matous polyposis (FAP) or hereditary non-polyposis cancer (HNPCC) syndromes, respectively (3,4).

Chemoprevention of these epithelial organ site cancers of breast and colon, using synthetic pharmacological agents or naturally occurring compounds individually or in combination to inhibit, reduce or delay the multi-step carcinogenic process has attracted intense clinical interest. Thus, the ability of chemo-preventive agents at low non-toxic levels to target early occurring carcinogenic events represents a major promise of current clinical preventive intervention (18,19).

The present review summarizes the data generated from our experiments designed to i) develop cell culture models from genetically engineered mice predisposed to breast and colon cancer, ii) identify specific and sensitive surrogate endpoint biomarkers for loss of homeostatic growth control and gain of carcinogenic risk, and iii) validate the developed cell culture based approaches as rapid screens for efficacious preventive/therapeutic agents.

2. Preclinical cell culture models for genetically predisposed breast and colon cancer

Breast. Aberrant expression of select oncogenes such as Ras, myc or HER-2 has been detected in several clinical organ site cancers including breast cancer (2,17-19). Similarly, targeted expression of these individual oncogenes in genetically engineered mice is associated with mammary carcinogenesis (5,15-17), and more importantly, stable transfection of these oncogenes in non-tumorigenic mammary epithelial cells is sufficient to induce multi-step tumorigenic transformation (20-22). With an objective of developing a cell culture model for oncogene-induced mammary carcinogenesis, we established stable transfectants from immortalized non-tumorigenic mouse mammary epithelial cells (MMEC) that were transfected independently with EJ-Ras and c-myc oncogenes and charac-terized the resultant MMEC-Ras and MMEC-myc cell lines (20,21).

Colon. Germ line mutations in the APC tumor suppressor gene represent a primary genetic defect in the FAP syndrome (3,4), while those in select DNA mismatch repair genes such as Mlh_1 represent a primary genetic defect in the HNPCC syndrome (4). Both these syndromes are associated with genetic predisposition for early onset familial/hereditary colon cancer (15,16). Genetically engineered mice carrying Apc and/or Apc plus DNA mismatch gene mutations exhibit high risk for

Table II. Biomarkers for aberrant proliferation and carcinogenic risk in mutant colon epithelial cells.

Biomarker	Cell Line				
	C57 COL	850[Min] COL	1638N COL	Mlh_1 COL	Mlh_1/1638N COL
Population doubling[a]	34	16	17	29	20
$S+G_2/M$: $subG_0$[b]	5.7±0.3	31.9±6.2	29.9±1.2	4.7±1.2	11.1±1.2
Anchorage-independent colonies[c]	0.8±0.5	18.9±2.5	17.7±2.3	0.6±0.3	13.7±1.8
Tumorigenicity[d]	0/10	10/10	10/10	0/10	8/10
Tumor latency	24 weeks	5-8 weeks	8-10 weeks	24 weeks	10-14 weeks

[a]Determined at the exponential growth phase, [b]determined by flow cytometry, [c]average number of colonies at day 14 post seeding, [d]determined by sub-cutaneous transplantation.

Table III. Selective efficacy of chemo-preventive test compounds in mammary and colon epithelial cells.

Mammary epithelial cell line	Inhibitory concentration $(IC_{50}\,\mu M)$[a]	
	4-HPR	TAM
MMEC	7.9	16.7
MMEC-Ras	2.8	6.2
MMEC-Myc	2.5	4.7
Colon epithelial cell line	SUL	DFMO
C57 COL	57.5	5.8
850[Min] COL	11.2	32.8
1638N COL	16.7	38.6
Mlh_1 COL	39.3	71.2
Mlh_1/1638N COL	14.3	35.7

[a]Extrapolated from cell viability at day 5 post-seeding from dose response of 5 log μM (0.01-100 μM) concentration range.

intestinal cancer (6-8,15,16). In an effort to develop preclinical *in vitro* colon epithelial cell culture models for FAP and HNPCC syndromes, we used histopathologically normal descending colon (a target organ site for colon cancer) from Apc mutant Apc850[Min]/+, Apc 1638N [+/-], and Mlh_1 [+/-]/1638N [+/-] mice to establish and characterize 850[Min] COL, 1638N COL and Mlh_1/1638N COL cell lines, respectively (23-26).

3. Biomarkers for carcinogenic risk

Loss of homeostatic growth control and gain of carcinogenic risk together characterize the pre-neoplastically transformed cells. Population doubling time, and status of proliferative and apoptotic cell populations represent the end point biomarkers for homeostatic growth control, while anchorage-independent growth and tumorigenecity represent the end point biomarker for carcinogenic risk.

Breast. The data generated from the experiments on mamary epithelial cells MMEC, MMEC-Ras and MMEC-myc are summarized in Table I. These data demonstrate that oncogene transfected cell lines exhibit 23.7-31.7% decrease in the population doubling time, relative to the parental MMEC cells. Furthermore, these transfected cells also exhibit 4.0- to 4.4- fold increase in the ratio of proliferative $(S+G_2/M)$ versus apoptotic $(subG_0)$ cell population due to increased proliferation and decreased cellular apoptosis. These data provided evidence that aberrant proliferation and down-regulated apoptosis lead to the loss of homeostatic growth control in oncogene transfected mammary epithelial MMEC-Ras and MMEC-myc cells.

The Ras and myc oncogene transfected cells exhibit 53.4- to 55.0-fold increase in the number of anchorage-independent (AI) colonies and greater than 90% incidence of tumors, 3-5 weeks post-transplantation. In contrast, the parental MMEC cells lack anchorage-independent colony formation and tumorigenicity. These data are consistent with several previous publications (20-22) and provide evidence for gain of carcinogenic risk in the oncogene transfected mammary epithelial cells.

Colon. The data generated in the cell lines C57 COL (Apc [+/+]/ Mlh_1 [+/+]), 850[Min] COL, 1638N COL (Apc [+/-], FAP models), and Mlh_1 COL and Mlh_1/1638N COL (Mlh_1[+/-], Apc [+/-], HNPCC model) are summarized in Table II. The cell culture models for FAP syndrome exhibit 50 to 65% decrease in the population doubling time and 4.2- to 12.7-fold increase in the $S+G_2/M$: $subG_0$ ratio due to aberrant proliferation and down-regulated apoptosis. In the cell culture model for the HNPCC syndrome these end points exhibit 41.2% decrease and 94.7% increase, respectively. It was noteworthy that in contrast to the double mutant Mlh_1/1638N COL cells, the Mlh_1 COL cells containing only the Mlh_1 mutation failed to express these perturbed biomarkers that are specific for the loss of homeostatic growth control.

Table IV. Modulation of cell cycle progression in oncogene transfected mammary epithelial cells.

Treatment	Concentration	G_1: S+G_2/M ratio[a]	
		MMEC-Ras	MMEC-Myc
EtOH	0.01%	0.6±0.2	0.7±0.3
4-HPR	1 μM	2.1±0.6[b]	5.4±0.7[e]
TAM	1 μM	7.3±0.8[c]	7.6±0.8[f]
4-HPR+TAM	1+1 μM	9.5±0.4[d]	12.2±0.6[g]

[a]Determined at day 5 post-seeding. Mean ± SD, n=6 per treatment group. [b-d, e-g]p=0.001; [c-d, f-g]p=0.03.

The anchorage-independent colony formation representing the surrogate end point biomarker for carcinogenic risk exhibited 19.7-31.1-fold increase in the number of AI colonies in the FAP models and 16.1-fold increase in the HNPCC model. The Mlh$_1$ COL cells did not exhibit anchorage-independent colony formation. The models for FAP and HNPCC syndromes also exhibited 80-100% incidence of tumors after a latent period of 8-14 weeks post-transplantation. In contrast, the normal C57 COL cells and Mlh$_1$ mutant Mlh$_1$ COL cells lacked the expression of the biomarker specific for carcinogenic risk. These data on Mlh$_1$ COL cells suggest that mutation in DNA repair gene alone is not sufficient to increase the carcinogenic risk.

These data taken together indicate that mutations in Apc alone or in both Apc and Mlh$_1$ genes together represent primary genetic defects that are responsible for induction of aberrant proliferation and for predisposition for enhanced carcinogenic risk in the present cell culture models of FAP and HNPCC syndromes, respectively. In contrast, mutation in the Mlh$_1$ gene alone may be insufficient to drive the carcinogenic process. It is noteworthy that mutations and/or other genetic defects in APC together with MLH$_1$ genes have been observed in clinical HNPCC (3,4,15,16).

4. Chemopreventive test compounds

Our previous publications have reported preventive efficacy of several mechanistically distinct pharmacological agents and naturally occurring phytochemicals on developed cell culture model for ER$^-$/PR$^-$/HER-2$^+$ comedo DCIS that is at risk for developing chemo-endocrine therapy resistant breast cancer (27-30), and on cell culture models for FAP syndrome (23-25) and HNPCC syndrome (26). These test compounds used individually at pharmacologically achievable dose levels, inhibit cell cycle progression, induce cellular apoptosis and reduce carcinogenic risk in all the cell culture models. It is noteworthy that the chemopreventive test compounds selected for present studies have documented evidence of efficacy both as single agents, as well as low dose combinations in the animal model systems and in clinical setting (31-36).

Breast. The experiments designed to examine the growth inhibitory effects of selected chemo-preventive test compounds on the ER$^+$ Ras and myc oncogene transfected mammary
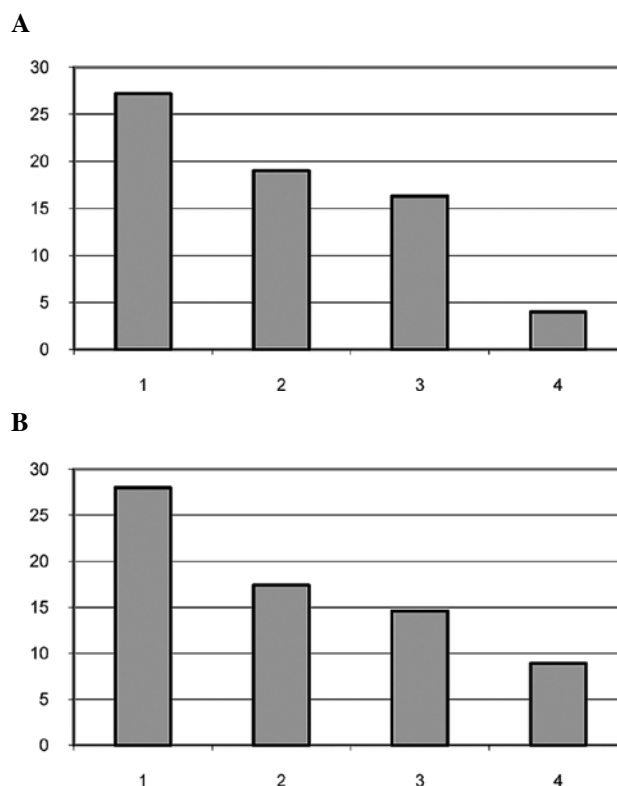
A

B

Figure 1. (A and B) Efficacy of 1 μM 4-HPR+1 μM TAM in combination on MMEC-Ras, and MMEC-Myc cells. (A) Anchorage independent colony formation in MMEC-Ras cells. Number of anchorage independent colonies, mean ± SD, n=12 per treatment group. (1) EtOH control, (2) 4-HPR, (3) TAM, (4) 4-HPR+TAM. Control versus combination p=0.001, single agent versus combination p=0.01. (B) Anchorage independent colony formation in MMEC-Myc cells. Number of anchorage independent colonies, mean ± SD, n=12 per treatment group. (1) EtOH control, (2) 4-HPR, (3) TAM, (4) 4-HPR+TAM. Control versus combination p=0.003, single agent versus combination p=0.01. Note that the combination induces greater reduction in the number of anchorage independent colonies relative to that induced by these agents alone (42).

epithelial cell culture models are summarized in Table III. Clinically relevant synthetic retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) and selective estrogen receptor modulator Tamoxifen (TAM) were selected as the test compounds for the experiments with mammary epithelial cell lines.

The synthetic retinoid 4-HPR functions as a potent inhibitor of reactive oxygen species and has documented efficacy against breast cancers irrespective of their hormone receptor status (31). The selective estrogen receptor modulator TAM has documented selective efficacy against estrogen receptor positive breast cancer (9). It was noteworthy that the oncogene transfected cells exhibited 63-94% greater sensitivity to growth inhibition relative to that exhibited by the parental cells (Table III).

Colon. Clinically relevant non-steriodal anti-inflammatory drug Sulindac (SUL) and selective orinithine decarboxylase inhibitor Difluoro methylornithine (DFMO) were selected as the test compounds for the experiments with the colon epithelial cell lines (Table III).

The synthetic non-steroidal anti-inflammatory drug, sulindac (SUL), functions as a pan-selective cyclo-oxygenase inhibitor,

Table V. Modulation of cell cycle progression in mutant colon epithelial cells.

Treatment	Concentration	G_1: $S+G_2/M$ ratio[a]		
		850[Min] COL	1638N COL	Mlh$_1$/1638N COL
EtOH	0.01%	3.1±0.7	3.3±0.4	2.7±0.6
SUL	2 μM	5.4±0.3[b]	10.5±0.6[e]	3.6±0.3[h]
DFMO	10 μM	8.2±0.8[c]	9.6±0.6[f]	6.8±0.6[i]
SUL+DFMO	2+10 μM	12.8±0.2[d]	15.3±1.0[g]	9.6±0.5[j]

[a]Determined at day 5 post-seeding. Mean ± SD, n=6 per treatment group. [b-d, i-j]p=0.001; [c-d, e-g, f-g, h-j]p=0.04.

and has documented efficacy in the FAP syndrome, as well as in sporadic colon cancer (32). The synthetic pharmacological agent difluoro-methylornitine (DFMO) functions as selective inhibitor of ornithine decarboxylase, a critical rate limiting enzyme in polyamine bio-synthetic pathway (33). Cyclo-oxygenase and ornithine decarboxylase enzymes are up-regulated in colon cancer and therefore, represent important molecular targets for preventive/therapeutic interventions (18,19,32,33).

The colon epithelial cell lines exhibiting genetic defects in Apc alone or in Apc and Mlh$_1$ represent relevant cell culture models for the FAP and HNPCC syndromes, respectively. These models exhibited 49.1-80.5% greater sensitivity to the test compounds relative to that observed in the C57 COL cell line. In this context, it was noteworthy that consistent with a lack of carcinogenic risk, Mlh$_1$ COL cells that have mutation only in the Mlh$_1$ DNA mismatch repair gene were substantially less sensitive to the inhibitory effects of the test compounds, than were the models for FAP and HNPCC syndromes.

5. Combinatorial preventive efficacy

Several recent reports have demonstrated that low-dose combinations of mechanistically distinct chemo-preventive agents exhibit enhanced efficacy relative to that produced by individual agents at comparative concentrations (34-36).

Breast. To examine the concept of combinatorial preventive efficacy in the developed mammary epithelial cell culture models, low dose combinations of 4-HPR+TAM were used on the oncogene transfected MMEC-Ras and MMEC-myc cells. The efficacy of the combinations was monitored by the status of G_1: $S+G_2/M$ ratio (specific for aberrant proliferation) and anchorage independent colony formation (specific for carcinogenic risk).

The data generated from the experiments designed to examine the effect of low dose combination of 4-HPR+TAM on G_1: $S+G_2/M$ ratio in MMEC-Ras and MMEC-Myc cells are presented in Table IV. These data demonstrate that in MMEC-Ras cells the 4-HPR+TAM combination is about 3.5-fold and 30% more efficacious in increasing the G_1: $S+G_2/M$ ratio relative to that observed by 4-HPR or TAM individually at equimolar concentrations. Similarly, the experiment on MMEC-Myc cells revealed a 1.2-fold and 60.5% greater efficacy of the combination relative to individual agents.

The data from the experiments designed to examine the effect of the 4-HPR+TAM combination on anchorage-independent colony formation in two oncogene transfected cell lines are presented in Fig. 1A and B. The AI colony number In MMEC-Ras cells treated with 4-HPR, TAM and 4-HPR+TAM was 19.0±2.8, 16.3±2.4 and 4.0±0.6, respectively. Thus, in these cells the combination exhibits about 77% greater efficacy in reduction of AI colonies relative to that observed by these agents individually at equimolar concentrations. The AI colony number in MMEC-Myc cells treated with 4-HPR, TAM and 4-HPR+TAM was 17.4±1.2, 14.6±1.0 and 8.9±0.6, respectively. These data indicated that 4-HPR+TAM combination exhibits about 44% greater efficacy relative to these agents individually.

Colon. To examine the concept of combinatorial preventive efficacy in the FAP and HNPCC models low-dose combination of SUL+DFMO were utilized. The data generated from experiments to examine the modulation in G_1: $S+G_2/M$ ratio by the low dose combination of SUL+DFMO are presented in Table V. In 850[Min] COL cells the combination of SUL+DFMO exhibit 1.4-fold and 56.1% greater efficacy to increase the G_1:$S+G_2/M$ ratio relative to that observed by these agents individually. In 1638N COL cells the combination exhibits 45.7% and 59.4% greater efficacy, while in Mlh$_1$/1638N COL cells the combination exhibits 1.7-fold and 41.2% greater efficacy.

The data from experiments designed to examine the effect of the SUL+DFMO combination on anchorage-independent colony formation in the three colon epithelial cell lines are presented in Fig. 2A-C. In 850[Min] COL cells the AI colony number in SUL, DFMO and SUL+DFMO treated groups was 14.4±2.4, 13.5±2.2 and 8.4±1.4, respectively. These data indicate that the combination exhibits about 39.7% greater efficacy in reduction of AI colonies. In 1638N COL cells the AI colony number SUL, DFMO and SUL+DFMO treated groups was 15.7±2.0, 14.2±1.8 and 9.8±1.3, while that in Mlh$_1$/1638N COL cells was 10.9±1.3, 8.9±1.1 and 6.0±0.7, respectively. Collectively, these data indicated that in the two cell lines the SUL+DFMO combination exhibits 41.2% and 38.7% greater efficacy respectively relative to these agents individually.

6. Conclusions and future directions

Preclinical models derived from genetically engineered mice represent valuable predictive models for human organ site
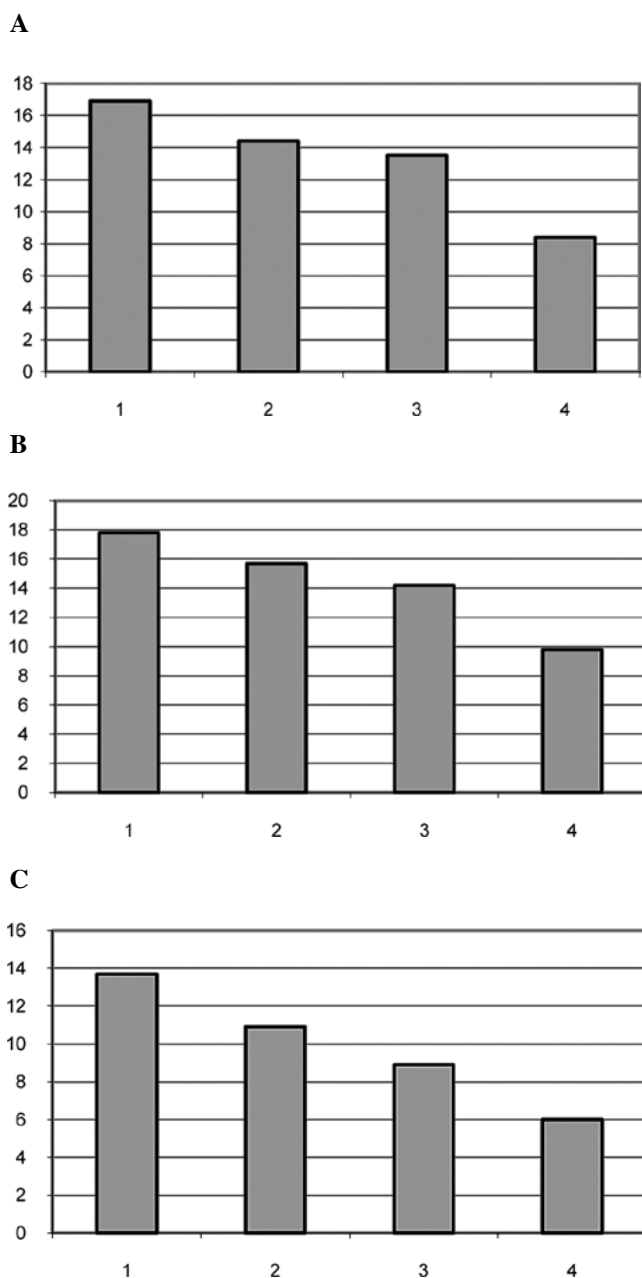
A

B

C

Figure 2. (A-C) Efficacy of 2 μM SUL+10 μM DFMO in combination on 850^{Min} COL, 1638N COL and Mlh$_1$/1638N COL cells. (A) Anchorage-independent colony formation in 850^{Min} COL cells, number of anchorage-independent colonies, mean ± SD, n=12 per treatment group. (1) EtOH control, (2) SUL, (3) DFMO, (4) SUL+DFMO. Control versus combination p=0.002. Single agent versus combination p=0.03. (B) Anchorage-independent colony formation in 1638N COL cells. Number of anchorage-independent colonies, mean ± SD, n=12 per treatment group. (1) EtOH control, (2) SUL, (3) DFMO, (4) SUL+DFMO. Control versus combination p=0.002, single agent versus combination p=0.03. (C) Anchorage-independent colony formation in Mlh$_1$/1638N COL cells. Number of anchorage-independent colonies, mean ± SD n=12 per treatment group. (1) EtOH control, (2) SUL, (3) DFMO, (4) SUL+DFMO. Control versus combination p=0.002, single agent versus combination p=0.03. Note that the combination induces greater reduction in the number of anchorage-independent colonies relative to that induced by these agents alone (42).

cancer. Target tissue derived epithelial cell lines expressing clinically relevant genetic defects and exhibiting quantifiable risk for carcinogenesis promise to provide facile experimental approaches that complement the existing animal models.

The data presented for mammary epithelial cell culture models demonstrate that targeted expression of Ras and Myc oncogenes drives the multi-step carcinogenic process by inducing aberrant cell cycle progression, down-regulated cellular apoptosis and anchorage-independent growth *in vitro* prior to tumor development *in vivo*. These modulations taken together provide evidence for oncogene mediated loss of homeostatic growth control and gain of carcinogenic risk. Essentially similar modulations in these surrogate end point biomarkers for carcinogenic risk have also been observed in the colonic epithelial cells exhibiting germ line mutations in the tumor suppressor Apc gene and/or in Apc plus DNA mismatch repair genes. The present biological characterization of the newly developed cell culture models now sets the stage for their molecular characterization relevant to the status of Ras, Myc, Apc signaling cascades and DNA repair pathways.

The experiments on the growth inhibitory effects of prototypical chemopreventive test compounds have provided evidence that preneoplastically transformed cells are more sensitive to growth inhibition than the isogenic parental non-transformed cells. The selectivity for chemopreventive efficacy validates these isogenic models as a unique approach for evaluation of mechanistic efficacy of new compounds.

Clinically relevant, mechanistically distinct 4-HPR and TAM for mammary epithelial cell culture models, and SUL and DFMO for colon epithelial cell culture models validate the clinical relevance of the present experimental approach, since these agents have documented preventive/therapeutic efficacy as single agents for clinical breast and colon cancer, respectively (9,12,13,18,19,31-33). Enhanced efficacy of these test compounds at low-dose combinations essentially extends and confirms similar efficacy observed *in vivo* in the genetically engineered mouse models (16,35,36). The proof of principle evidence obtained from the data on combinatorial preventive efficacy now provides a scientifically robust basis for future mechanistic studies to identify molecular targets critical for enhanced efficacy. Furthermore, these data also validate the present experimental approaches for rapid prioritization of new chemopreventive agents for future animal studies and subsequent clinical trials. In this context it is noteworthy that combinatorial efficacy of SUL+DFMO has been recently reported in a clinical trial on sporadic colon cancer (34).

Recent evidence suggests that cancer initiating stem cells representing therapy resistant minor population may be responsible for frequent acquired tumor resistance to long-term chemo-endocrine therapy in several heterogeneous epithelial organ site cancers (37). Established human breast carcinoma cell lines, such as MCF-7 and T47D as well as clinical breast cancer specimens, exhibit the presence of cancer initiating stem cell population (37,38). Normal target organs for these cancers also exhibit the presence of self renewing, pluripotent stem cell population (39). Recent research on clinical pancreatic adenocarcinoma specimens has demonstrated the presence of CD44$^{+/High}$/CD24$^{-/Low}$/ESA enriched stem cells exhibiting the property of self-renewal, ability for production of differentiated progeny and accelerated tumor development (40), and abrogation of stem cell characteristics via synergistic efficacy of a combination of a synthetic multi-kinase inhibitor sorafenib with a naturally occurring NF-κB inhibitor sulforaphane (41).

The data generated for the breast epithelial cell lines (20,21), and for the colon epithelial cell lines (23-25) has been recently summarized (42). Thus, in the present context, availability of well characterized breast epithelial cell lines MMEC-Ras and MMEC-Myc, and colon epithelial cell lines 850^{Min} COL, 1638N COL and Mlh$_1$/1638N COL may represent ideal cell culture systems to generate cancer initiating stem cells and to evaluate their responses to conventional chemo-endocrine therapy. Such unique models promise to identify novel preventive/therapeutic interventions targeted specifically towards therapy resistant carcinogenic stem cell populations.

Acknowledgements

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