Published in final edited form as:

Mutat Res. 2009 November 2; 670(1-2): 96–98. doi:10.1016/j.mrfmmm.2009.06.011.

Genetic Pathways to Colorectal Cancer

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Abstract

The colorectal cancer paradigm explains how genetic and histological changes lead normal epithelial cell to transform into pre-malignant adenomas then progress to malignant carcinomas. Using the Genetic Alterations in Cancer Knowledge System intragenic allele loss and gene mutation data from approximately 9000 colorectal tumors were compared to the model of colorectal tumor development. The distribution of mutations along the TP53 codons as a function of tumorigenesis also was analyzed. Alterations of APC, KRAS and TP53 were observed in a higher percentage of adenocarcinomas compared to adenomas ($P < 0.05$) indicating that the alterations accumulated with malignancy. Alterations in BRAF, CTNNB, HRAS and NRAS were infrequent regardless of morphology. Differences were observed in the distribution of TP53 mutations with tumorigenesis. Mutations (single base substitutions) occurred most frequently at codons 175 and 273 in both tumor types; however, in adenocarcinomas the mutation incidence at codon 248 was approximately three times that reported in adenomas. It is proposed that the higher incidence of mutation at codon 248 is a later event in colorectal tumorigenesis that occurs as the tumors become malignant.

1. Introduction

Cancers are a complex group of diseases caused by both environmental and heritable factors (1,2). Considerable research efforts are focused on defining the acquired genetic changes that dysregulate signaling and functional pathways and lead to malignancy. Rarely though are cancers the result of a single exposure or factor, making carcinogenesis a complex problem in which ill-defined exposures and genetic constitutions lead to multiple types of genetic damage and modification of multiple genetic pathways (3). The colorectal cancer (CRC) paradigm described by Fearon and Vogelstein (4) illustrates how concurrent genetic and histological changes lead normal epithelial cells to become benign adenomas and malignant carcinomas. Recent examination of the colorectal cancer genome of malignant tumors suggests that as many as 20 cancer-associated genes may play a role in this process (5,6). Still it is evident that it is not individual genes that govern the course of tumorigenesis but pathways that lead to a deregulation of net cell growth (7). Underlying the initiation of CRC development is WNT/APC/ β -catenin pathway mutation and dysregulation, this is followed by activation of the RAS-RAF-MAPK signaling pathway as adenomas grow and progress. Alteration of the TGF β signal transduction pathway induces adenomas to become early carcinomas with the final transition

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Conflict of Interest Statement The authors declare that there are no conflicts of interest.

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of a benign to a malignant tumor being characterized by TP53 alterations (7,8). While these events describe one major pathway to colorectal cancer development, they do not explain tumor formation and progression in its entirety. Evidence shows chromosome gains at 8q, 13q and 20q and losses at 8p, 15q, 17p and 18q are also strongly associated with the progression of adenomas to carcinomas (9). Also CpG island hypo- or hypermethylation in specific gene sequences can induce gene silencing or loss of imprinting in tumor suppressor genes, DNA repair genes or cell cycle regulatory genes (for example: IGF2, CDKN2A, APC, BRAC1, and MLH1) resulting in deregulation of cell growth (10).

To explore how the literature reflects changes in genetic pathways we have analyzed gene alteration data from published literature for a large series of approximately 9000 colorectal tumors and correlated these findings with tumor morphology.

2. Materials and Methods

2.1. Data Selection

Mutation data was obtained from the Genetic Alterations in Cancer (GAC) Knowledge System (<http://www.niehs.nih.gov/research/resources/databases/gac/index.cfm>) which was last updated May 2009. A complete description of the GAC database has been published previously (11). Briefly data from studies of gene alterations in human colorectal tumors from individuals were retrieved from the database using the data mining feature. The database query was defined by the parameters human, primary, colorectal, adenomas or adenocarcinomas with unknown etiology (spontaneous). Data were reported for mutations (missense, nonsense and silent point mutations, frameshift insertions and deletions, in-frame insertions and deletions) or loss of heterozygosity (single allele loss of intragenic markers or markers tightly linked to genes) and represented somatic alterations only arising in both sporadic and hereditary tumors. Germ line mutations were excluded based on authors' comparisons of tumor and constitutional DNA. Results are presented as separate datasets; bi-allelic alterations in single tumors are not shown.

2.2. Statistical Analysis

To compare the incidences of alterations, detailed data showing the results for each tumor sample from all studies (Supplementary tables 1 and 2) were generated by the GAC system and prepared for broad-based analysis. The mutation and loss of heterozygosity (LOH) incidences were compared for pairs of data groups (adenoma versus adenocarcinomas) however since samples were derived from multiple studies, a non-parametric estimator of variability was used (12). Analysis was performed using the proc multtest with a bootstrap adjustment to generate the p values (13).

3. Results and Discussion

Analysis of gene alteration data from the GAC Knowledge System was performed to evaluate the incidences of mutation and allele loss for nine cancer genes in approximately 9000 colorectal tumors reported from 149 peer-reviews publications. Evaluation was performed as a function of tumor morphology to assess how genetic pathways changed with malignancy. In more than 20% of adenomas gene mutation and allele loss occurred in APC, KRAS, DCC and MCC (Figure 1A, B). Each of these genes has been proposed to play a role in the initiation of CRC development and formation of adenomas. APC, which forms part of the WNT pathway that plays an important role in tumor initiation, had the highest overall incidence of alterations in adenomas: 40% (443/1119) mutations and 18% (19/107) LOH. KRAS, DCC and MCC also contributed to adenoma growth and progression; KRAS mutations were found in 19% (460/2385) of tumors, 20% of tumors examined had LOH in DCC and MCC. Inactivation of

TP53 was uncommon in adenomas; only 7% (28/422) had mutations and 17% (5/30) had allele loss.

Malignant tumors had a higher frequency of altered genes compared to adenomas suggesting accumulation of mutations and hypermutation frequencies play a key role in malignancy. The APC, KRAS and TP53 genes all had significantly higher incidence of alterations in adenocarcinomas than in adenomas ($P < 0.05$, Figure 1A, B). In the APC gene allelic loss occurred in 37% (174/467) of the tumors, in KRAS the activating events were mutations (34% (1058/3111) of tumors). Significant increase in both mutations and LOH (7 to 43% mutations, 17 to 58% LOH) were observed in TP53. Additionally, DCC LOH occurred more frequently in adenocarcinomas (44%) than adenomas (20%). The DCC, KRAS and TP53 genes all contribute to adenoma progression (KRAS and DCC) and malignant transformation (TP53). Loss of APC gene function is considered one of the initiating events in CRC formation (14); results presented here show that alterations to APC continue to accumulate with malignancy. This may indicate that APC plays a role in tumor progression. Alterations in other genes analyzed: BRAF, CTNNB1, HRAS and NRAS, were infrequent ($< 10\%$) in both tumor groups and did not vary significantly during tumor progression. This suggests that although alterations in these genes may confer a growth advantage on some individual tumors, they do not appear to play an integral part in CRC tumorigenesis.

With the progression to malignancy, TP53 was the gene in which the greatest increase in inactivation was seen. To establish whether quantitative changes in mutation incidence were mirrored by qualitative changes in mutation type we analyzed TP53 mutation spectra and distribution (Figure 2). The most common types of substitutions reported were GC>AT transitions in both adenomas and adenocarcinomas (57% in adenomas and 53% in adenocarcinomas). Other types of substitutions occurred much less frequently ($\leq 10\%$ of tumors) regardless of tumor type. The distribution of TP53 mutations along the gene codons however differed between the pre-malignant and malignant tumors (Figure 2A, B). In adenocarcinomas the highest incidence of mutations were observed at codon 175, 248 and 273 ($\sim 12\%$) but in the adenomas only codons 175 and 273 had the higher incidence (~ 14 and 10% , respectively). The mutation incidence at codon 248 ($\sim 4\%$) was three times lower than that in the adenocarcinomas. In both cases the mutations were almost exclusively GC>AT transitions at CpG dinucleotides (Supplementary table 3). It has been suggested that in adenocarcinomas the location of the mutational hot spots is in part determined by the bias of the substitution spectrum for GC>AT at CpG sites. What causes the difference in the distribution of the most frequently mutated codons is not clear; perhaps it is a reflection of change in epigenetic modification of the TP53 during carcinogenesis. In 1995, Tornaletti and Pfeifer showed that in normal colon mucosal cells, all potential CpG sites in exons 5–8 (codons 126–306) of the TP53 gene were methylated (15). Mutations at CpG sites are common in CRC and are caused by the endogenous deamination of 5-methylcytosine rather than by environmental exposures. Changes in methylation patterns might therefore have the potential to affect the distribution of mutations within the gene. Alterations in methylation patterns concurrent with tumor progression have been observed previously (16,17,18) but how these trends are reflected in the methylation of specific TP53 CpG sites, such as codon 248, is unknown.

In conclusion, analysis of the data from ~ 9000 tumors supports the current model of CRC and demonstrates that accumulation of mutations in genes such as APC, KRAS and TP53 can deregulate distinct genetic pathways and lead to cancer development. With the progression to malignancy the incidence of mutation in specific TP53 codons at which mutations most frequently occurred changed indicating that differential epigenetic modification of TP53 may occur during carcinogenesis, an observation that requires further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences [contract number N43-ES-15477].

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Figure 1A.

Incidence of somatic mutations (point mutations and in-frame and frameshift insertions or deletions) in colorectal adenomas (gray) and adenocarcinomas (black). Detailed information for these datasets is given in supplementary table 1. * $P < 0.05$.

Figure 1B.

Incidence of loss of heterozygosity at intragenic markers or markers tightly linked to specific genes in colorectal adenomas (gray) and adenocarcinomas (black). Detailed information for these datasets is given in supplementary table 2. * $P < 0.05$.

Figure 2.

The percentage of single base substitutions in individual codons of TP53 from (A) 422 adenomas and (B) 1379 adenocarcinomas.