East African Medical Journal Vol. 78 No. 7 (Supplement) July 2001
LESSONS LEARNED FROM COLORECTAL MODEL OF TUMOURIGENESIS
J. Wakhisi, PhD, Senior Lecturer, Department of Medical Biochemistry, Faculty of Health Sciences, Moi University, P.O. Box 3900, Eldoret, Kenya.

LESSONS LEARNED FROM COLORECTAL MODEL OF TUMOURIGENESIS

J. WAKHISI

ABSTRACT

Genetic analytical techniques were carried out to identify mutations in adenomatous polyposis coli (APC) gene and K-ras oncogene in colorectal tumourigenesis. These two genes are said to be early mutation genes among other mutation genes that constitute the model for colorectal tumourigenesis. To do this analysis, DNA was isolated from colorectal formalin fixed paraffin-embedded tumour tissue sections. The sections were deparaffinised, digested in proteinase-K, followed by DNA isolation. The DNA was amplified by Polymerase Chain Reaction (PCR), screened by using Denaturing Gradient Gel Electrophoresis (DGGE) or Single Strand Conformation Polymorphism (SSCP) and then sequenced. These results lend support to the fact that colorectal cancer and indeed cancer in general develops through a multi-step process; also that accumulation of genetic mutations underlie the development of neoplasia. We are in the process of extending this study to cancer of oesophagus to see if a similar or parallel model of carcinogenesis holds and in what sequence it is.

INTRODUCTION

The abnormalities of cancer cells are based on genetic change and this is accomplished by altered genes which have been observed to be central mediators in carcinogenesis. This reinforces the notion that no matter what form cancer takes, it remains a malady of genes. Most, if not, all causes of cancer act by damaging genes directly or indirectly. The genetic apparatus is at fault in tumourigenesis. It has also been observed for long that the onset of cancer is a gradual stepwise process that may unfold over the course of decades rather than a single fixed event. It is now clear that for a normal cell to evolve into a cancer cell multiple heritable changes within the cell are required, that is, carcinogenesis is a multiple (multistage) process involving multiple genes. This is because in most multi-step (multistage) models of carcinogenesis genetic and or epigenetic alterations of multiple, independent genes are involved. Carcinogenesis is a multi-step process at both the phenotypic and genetic levels.

A malignant neoplasm has several phenotypic attributes, such as excessive uncontrolled growth, local invasiveness and the ability to form distant metastases. These characteristics are acquired in a stepwise fashion, a phenomenon called tumour progression. At molecular level progression results from accumulation of genetic lessons that in some instances are favoured by defects in DNA repair. Thus the multiple individual steps in tumourigenesis may be nothing more than the accretion of genetic lesions involving several genes with the sum being a malignant cell.

Two gene classes, which together constitute only a small proportion of the full genetic set play major roles in triggering cancer. In their configuration, they choreograph the life cycle; of the cell the intricate sequence of events by which a cell enlarges and divides. These are protoncogenes and tumour suppressor genes to which we can add genes that regulate programmed cell death (apoptosis) and genes that regulate repair of damaged DNA.

Protoncogenes encourage growth (growth promoting), whereas tumour suppressor genes inhibit growth (growth inhibiting). Collectively these two gene classes account for much of the uncontrolled cell proliferation seen in human cancers. When mutated, protoncogenes can become carcinogenic oncogenes that drive excessive cell multiplication. The mutations may cause the protoncogene to yield too much of its encoded growth stimulatory protein or an overly active form of it. Tumour suppressor genes, in contrast contribute to cancer when they are inactivated by mutations. The resulting loss of functional suppressor proteins deprives the cell of crucial brakes that prevent inappropriate growth.

For a cancerous tumour to develop, mutations must occur in half a dozen or more of the founding cell growth controlling genes. Altered forms of yet other classes of genes may also participate in the creation of a malignancy, by specifically enabling a proliferating cell to become invasive or capable of spreading (metastasizing) throughout the body.

We carried out a mutation analysis in two gene; one a proto-oncogene and another a tumour suppressor gene i.e K-ras gene and APC gene respectively. These two genes among others play important roles early in colorectal carcinogenesis.

MATERIALS AND METHODS

From the formalin-fixed, paraffin-embedded tissues, a 5um section of each tumour sample was cut and mounted on a slide to be stained with haematoxylin and eosin (H&E) staining. Several

(10) 10um sections were then cut parallel to the H&E section. Tumour areas containing neoplastic cells as identified by the H&E stained section, were carefully scraped off and put into a sterile microfuge tube. The pellets, after deparaffination with xylene and dehydration with ethanol, were incubated with proteinase-k and digested over night. The yield and purity of DNA was determined by measuring its concentration at a wavelength of 260 n.m.

APC gene: DNA amplification of APC gene was carried out on two overlapping fragments spanning the mutation cluster region (MCR). This resulted in two fragments A and B. Fragment A, starting at nucleotide 3874 and ending at nucleotide 4229 and fragment B from nucleotide 4114 to 4624. The amplified PCR products were separated on a 2% agarose gel to check the results of amplification. Screening for mutations in APC gene on the PCR products was carried out by Denaturing Gradient Gel Electrophoresis (DGGE). PCR products showing mutation on (DGGE), were sequenced using the Big Gye Terminator Cycle sequencing Ready Reaction Kit on Perkin Elmer Fluorescent sequencer.

K-ras gene: For single strand conformation polymorphism (SSCP) analysis, the DNA was amplified in two steps to obtain a specific polymerase. Chain Reaction (PCR) product. Screening for mutation in K-ras gene of the PCR product was carried out by SSCP. PCR products showing mutation on SSCP analysis were sequenced to identify the mutation.

RESULTS

K-ras gene is located on the short arm of human chromosome 12. Most of K-ras mutations occurred in codon 12 but a few mutations also occurred in codon 13 and 61. Various mutation types were observed such as G-T transversions and G-C transversions as well as G-A transitions. Most of the mutated adenomas showed G-T transversions located particularly in the first base of codon 12 G-c transversions were seen only in Dukes D stage, whereas G-T transversions were most common in all stages except Dukes D.

APC gene is located on human chromosome 5q 21-22. It has a coding sequence of 8535 base pairs in 15 exons. Approximately 75% of the somatic mutations occurred in the Mutation Cluster Region (MCR) between codons 1280 and 1500. There was heterogenous pattern of the mutations. Approximately 50% were point mutations. 43% deletions, and 7% were insertions.

DISCUSSION

Approximately 30% of all human cancers contain mutated versions of the ras gene. In colon cancer, the incidence of ras mutations is even higher. Indeed mutations of the ras gene is the single most common oncogen abnormality in human tumours.

The ras family of protein binds guanosine triphosphate (GTP) and guanosine diphosphate (GDP). Normal ras proteins flip back and forth between an excited signal-transmitting state and quiescent state. In the inactive state ras proteins bind GDP. When cells are stimulated by growth factors, inactive ras becomes activated by exchanging GDP for GTP. The activated ras in turn activated downstream regulators of proliferations, including several cytoplasmic kinases which flood the nucleus with signals for cell proliferation.

However, the excited signal emitting stage of the normal ras protein is short lived, because its intrinsic guanasine triphosphatase (GTPase) activity hydrolyses GTP to GDP thereby releasing a phosphate group and returning the protein to its quiescent ground state. The GTPase activity of the activated ras protein is dramatically magnified by a family of GTPase-activating protein (GAPa). Thus GAPs act as molecular "brakes" that prevent uncontrolled ras activation by favouring hydrolysis of GTP and GDP. Mutant ras proteins are trapped in their activated GTP-bound form and the cell is led to believe that it must continue to proliferate.

It follows from this scenario that the consequences of mutations in the ras protein would be mimicked by mutations in the GAPs that fail to restrain normal ras proteins.

APC gene: Despite the heterogenous character of the mutations at gene level 100% of the aberrations detected led to similar effects at protein level, that is, truncation of the APC protein due to translational termination.

The majority of APC gene mutations (>95%) described thus far result in truncation of its protein product with deletion of the carboxyl, portion. Inactivation of the APC protein by loss of the carboxyl terminus region is apparently important in adenoma development and may relate to its interaction with microtubule and catenin, as well as its effect on apoptosis. The detection of APC mutation in lesions as small as 5 mm suggest their involvement in a very early stage of tumourigenesis.

Research work elsewhere has charactised a more detailed genetic model for colorectal tumourigenesis. From this model, mutations in the APC tumour-suppressor gene occur early while mutations in K-ras oncogene are characteristic for the transition from early to intermediate adenomas. Loss of function of the deleted in colorectal cancer (DCC) gene contributes to intermediate and late adenoma step, while alterations of the P53 tumour suppressor gene are late events in carcinogenesis, occurring mainly from late adenoma to carcinoma. We would like to carry out a study to establish whether a similar model exists for cancer of oesophagus and in what gene-mutation order it exists.