Review Article

Cancers of the Upper Gastro-intestinal Tract: A Review of Somatic Mutation Distributions

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Abstract

Cancers of the upper gastro-intestinal tract (UGIT) comprise esophageal, esophago-gastric junction, stomach and duodenal cancers. Together, these cancers represent over 1.5 million cases and are the cause of about 1.25 million deaths annually. This group of cancers encompasses diseases with marked disparities in etiology, geographic distribution, histopathological features and frequency. Based on histological origin, squamous cell carcinoma of the esophagus (ESCC), which arises through a dysplasia-carcinoma sequence within the squamous mucosa, is a completely different cancer than junction, stomach and duodenal cancers, which develop within glandular epithelia through cascades involving inflammation, metaplasia, dysplasia and carcinoma. At the frontline between these two histological domains, cancers of the esophago-gastric junction constitute a mixed group of glandular tumors including distal esophageal adenocarcinomas and cancers arising within the most proximal part of the stomach - the cardia. Most of UGIT cancers are sporadic, although familial susceptibility genes have been identified for stomach and rare cases of ESCC. We have used the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) to identify genes commonly mutated in UGIT cancers. Regardless of etiology and histopathology, three genes are mutated in at least 5% of UGIT cancers: *TP53*, *CDKN2a* and *PIK3CA*. Another three genes, *NFE2L2*, *PTCH1* and *NOTCH1*, are mutated in ESCC only. Conversely, genes of the *RAS* family and of the *CDH1/APC/CTNNB1* pathway are mutated only in non-squamous cancers, with differences in mutated genes according to topography. We review the potential functional significance of these observations for understanding mechanisms of UGIT carcinogenesis.

Key words: Esogastric junction, mutations, upper GI tract cancers

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Introduction

Upper gastrointestinal tract includes the esophagus, stomach and the first part of small intestine, duodenum. Cancers arising from these organs are diverse in their topography, morphology, etiology and natural history. Worldwide, these cancers affect about 1.6M people annually, representing about 15% of all cancers. However, incidence rates show considerable variations among genders, populations, geographic areas, as well as significant temporal variations.[1]

Cancer of the esophagus accounts for about 0.5M annual cases, 68% of them occurring in males. They include two distinct morphological entities, squamous cell carcinoma (ESCC, originating from the squamous esophageal mucosa) and adenocarcinoma (EADC, originating from sub-mucosal glandular structure and from glandular metaplasia in the lower esophagus). ESCC is the primary cancer in most parts of the world with extremely variable incidence rates across geographic areas. In the past decades, rates of EADC have substantially increased in many industrialized countries.[2] Gastric (stomach) cancer (GC) accounts for over 1M annual cases, 65% of them occurring in males. They are subdivided according to their topology in the stomach (antrum, fundus, and cardia). For the purpose of this review, the most relevant distinction is between cardia (CGC) and non-cardia (NCGC) gastric cancers. CGC forms a group of malignancies originating from the junction of tubular esophagus with saccular stomach at the esophago-gastric junction, so called esophago-gastric junctional (EGJ) tumors. There is ongoing debate on histological, molecular and precise cell and tissue origin of tumors commonly grouped within EGJT.[3,4] NCGC is the 4th most common cancer and 2nd in terms of mortality, with a predominance in low-resource and emerging countries. In industrialized countries, it has been by far the main cause of cancer death until the 1950's, after which rates have dropped significantly, except in Japan.[5] Duodenal cancers (DC) are the most common cancers in the small intestine but do not rank among the 20th most common forms of cancer anywhere in the world.[1]

The molecular pathology and etiology of esophageal cancer, esophago-gastric and gastric cancers have been the topic of several synthetic reviews.[6–14] Here we will discuss their molecular features, using as starting point the analysis of their specific patterns of mutations. This analysis is based on The COSMIC database (Sanger Institute, UK) which compiles data from gene-specific and whole ge-

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nome/exome sequencing studies, providing a catalogue of genetic alterations in cancer diseases (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/), and on the *TP53* database maintained at IARC, France, which makes it possible to analyze variations in mutation types and frequencies within a single, frequently mutated tumor suppressor gene, *TP53* (http://www.p53.iarc.fr). Based on this information, we propose a simple model for stepwise transformation of progenitor cells of endodermal origin in the digestive tract into distinct morphological and histological pathways accounting for the diversity of UGIT cancers.

Definitions and risk factors of UGIT cancers

Most UGIT cancers have sporadic origin and their etiology is complex, combining dietary, environmental, infectious and metabolic risk factors. The heterogeneous geographic distribution of these cancers can be largely accounted for by variations in these factors and their combinations.

***ESCC*:** These cancers develop from the squamous esophageal mucosa through a typical dysplasia-carcinoma sequence. There is no defined precursor condition (considering dysplasia as pre-neoplastic or early neoplastic lesion rather than "precursor", fore-runner, to neoplasia).[15,16] Esophagitis, a common inflammatory condition characterized by structural changes and lymphoid cell infiltration, does not appear to be associated with a significant increase in risk of ESCC.[17] Exogenous risk factors include combined tobacco and alcohol addiction as well as, in many low-resource countries, consumption of scalding hot beverages, low vitamin-antioxidant levels associated by deprivation and low consumption of fresh fruits and vegetables.[18,19] Despite extensive studies, there is no evidence in support for a role of infectious agents such as Human Papilloma Viruses.[20–24] In Western Europe, incidences of ESCC were historically high in defined regions of alcohol production and high alcohol consumption, with an approximately 10-fold excess of cases in men. However, in recent years, changing trends have been noted, most likely in relation with changing patterns of tobacco and alcohol usage and with a decrease in male to female ratio.[25] In other parts of the world, areas of high incidence are generally rural, mountainous areas with limited resources and poor dietary diversity. In such areas, incidence rates are equally high in males and females. The highest incidences have been reported in population groups of Mongolian origin (Turkmens) in Northern Iran and in central China, as well as in parts of South-East Africa and Southern America.[18,26–28] Intervention studies using vitamin and oligo-element supplementation in China have shown limited but objective protective effects.[29,30]

***EADC*:** This cancer develops in the lower third of the esophagus from a precursor lesion, intestinal metaplasia, which is often associated with chronic mucosal damage caused by bile-acid gastro-esophageal reflux. Barrett's metaplasia is a specific form of intestinal metaplasia that can be interpreted as reactive tissue regeneration in patients with chronic Gastro-Esophageal Reflux Disease (GERD). The extent of Barrett's metaplasia is variable, from short segment (a few millimeters above the squamous-columnar junction) to long-segment (several centimeters, often with irregular, patchy pattern, above the junction). Factors promoting GERD include overweight, hiatal hernia and functional impairment of the lower esophageal sphincter.[31,32] In a systematic analysis of population attributable risk (PAR) for EADC in the United States, Engel *et al*. (2003) identified high body-mass index (BMI), GERD, smoking and low fruit and vegetable consumption as factors accounting for a combined PAR of 78.7% (95% CI = 66.5% to 87.3%).[33]

***CGC*:** Cardia Gastric Cancers are a group of adenocarcinoma defined by their topological origin at of within a few centimeters of the EGJ. Literature on EGJ is confusing because of the often-indiscriminate inclusion in this group of cancers originating from the upper stomach or from either glandular or columnar mucosal structures within the OGJ.[4,34] Tanière *et al.* (2001) have used absence of microscopic and macroscopic evidence of short-segment Barrett's mucosa as a criterion to distinguish cancers arising from the specific cardia mucosa from EADC arising within Barrett's metaplasia.[35] Derakhshan *et al*. (2008) have proposed another distinction, separating two types of cardia cancer based on their suspected etiologies. According to this distinction, one type of CGC may arise from severe atrophic gastritis and be of intestinal or diffuse subtype similar to non-cardia cancer, whereas another type may be related to GERD and intestinal in subtype, similar to EADC.[36] Overall, gastric atrophy, GERD symptoms and histological subtype may help distinguish between gastric versus esophageal origins of cardia cancer. CGC differs from both EADC and NCGC by the absence of known precursor and by distinct trends of incidence. Whereas NCGC has shown a tendency to decrease in incidence in several parts of the world, CGC has shown an opposite trend and is estimated to represent up to 30% of all GC cases.[37–40] The etiology is ill-defined, with particularly controversial involvement of *Helicobacter pylori (H. pylori)*. Recent studies have suggested that the proximal cardia region of the stomach may have less acidic buffering following meals than the more distal stomach. High-definition pH-metry showed that while the rest of the stomach shows a marked fall in acidity on ingesting a meal, the cardia increases in acidity to become the most acidic region throughout the postprandial period.[41] Thus, specific conditions in this region may lead to patterns of inflammation and mucosal damage and repair that differ from those occurring in the lower esophagus and distal stomach.

***NCGC*:** Cancers of the distal stomach mainly occur as two histological types defining the Lauren's classification, diffuse and intestinal types. The most common form is the intestinal type. Chronic gastritis followed by mucosal atrophy is a precursor condition. Main risk factors include chronic infection with *H. pylori*, leading to hypochlorhydria or achlorhydria, intestinal metaplasia and favoring the onset of

a dysplasia–carcinoma progression sequence.[42,43] Other risk factors include consumption of salty food and low levels of vitamins and antioxidants that favor the formation of mutagenic *N*-nitroso compounds.

The main defining criterion for the diffuse type is loss of expression of E-Cadherin, leading to a specific pattern of apparently loosely attached cells. Gastritis and intestinal metaplasia are not precursors for diffuse type. *H. pylori* infection is however a defined risk factor.[44–46]

Genetic predisposition

Most UGIT cancers are sporadic. High risk of ESCC is observed in families with Tylosis (diffuse nonepidermolytic palmoplantar keratoderma), a rare autosomal dominant condition present from infancy, characterized by a well-demarcated keratoderma involving the entire palms and soles.[47] Germline mutations in *RHBDF2* (17q25.1) encoding a serine protease associated with *EGFR* signaling have been identified in such families.[48] So far, there is no evidence that *RHBDH2* is a target for somatic mutation in sporadic ESCC. It has been hypothesized that ESCC might be more common into certain ethnic groups (e.g., groups with Mongolian ancestry in Central Asia).[18] However, so far, genetic evidence for this association is lacking. For GC, studies in the Sweden familial cancer registry have identified an increased risk in relative of patients with GC.[49] The most common inherited form of GC is the diffuse type, associated with inactivating germline mutation in *CDH1* (16q22.1) which encodes E-Cadherin. Rare occurrences of UGIT cancers have been reported as part of complex cancer patterns in several familial predisposition syndromes including Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer, HNPCC; GC) and Bloom's Syndrome or Dyskeratosis Congenita (ESCC).[50] Other familial cancers with UGIT localization include hamartomas in Peutz-Jeghers Syndrome (caused by mutations in *LKB1/STK11* (19p13.3) and GIST (Gastro-Intestinal Stromal Tumors).[50]

Genetic alteration in UGIT cancers

The genome of cancer cells undergoes rapid and complex genetic changes that underlie the process of progression from pre-neoplastic lesions to cancer and metastatic disease. These changes include targeted mutations (single base substitutions, insertions, deletions) that either activate or inactivate the function of specific genes, rearrangements, translocations, loss of alleles and amplifications that collectively modify the structure and activity of a number of genes within defined chromosomal regions, aneuploidy which results in gain or loss of entire chromosomes, and massive, multi-site genomic rearrangements such as chromothripsis, in which entire chromosome regions are scattered and pieced back together in an apparently random manner. In addition to these various degrees of genetic changes, multiple alterations occur at epigenetic level, encompassing changes in the methylation level of gene promoters (thus impacting on gene expression) and modifications in chromatin structure and access to coding regions by the replication, transcription and repair machineries. Collectively, these changes profoundly modify the patterns of gene expression (transcriptome), protein synthesis (proteome) and cell/tissue metabolic activities (metabolome). In this section, we are discussing only one aspect of these multiple changes: small-size mutations that alter the structure and function of specific genes. Indeed, there is good evidence that the nature of genes affected by mutation, as well as the types of mutations, may substantially vary by tumor type and etiology. Furthermore, data on mutations are accumulating as a result of large-scale sequencing efforts and there is a rapid growth in mutation data available in public databases such as the COSMIC database.

COSMIC compiles information on the position and nature of mutations in human cancer tissues. The most studied gene for mutations is the tumor suppressor gene *TP53*. As per June 2013, COSMIC contained information on *TP53* mutations in 1,858 ESCC, 542 EADC and 3442 GC (all types). For DC, the dataset was limited to studies on *KRAS* mutations (detected in 47 of 156 tested samples). In Table 1, we summarize the information available on the most commonly mutated genes in UGIT cancers. We have arbitrarily selected genes that were mutated in at least 3% of analyzed cases, and for which at least 50 cases had been analyzed. Results show that genes mutated in UGIT cancer fall into three groups. Group 1 includes three loci targeted at various frequencies by mutation in all UGIT cancers, irrespective of topography and morphology. This group includes *TP53*, *CDKN2a* and *PI3K3CA*. Group 2 includes genes that are specifically mutated in ESCC and not with cancer of glandular morphology. These genes are *NFE2L2*, *PTCH1* and *NOTCH1* (for which only 37 samples have been reported so far, with a mutation prevalence of 43%). Group 3 includes genes that are altered in UGIT cancers with glandular morphology, including members of the *RAS* family (*KRAS*, *NRAS*, *HRAS*) and genes involved in Wnt/BetaCatenin/Cadherin signaling pathway (*CTNNB1*, *APC*, *CDH1*). In addition to these categories based on distribution and frequency of mutations, two genes involved in Mismatch Repair (MMS) are detected as mutant in a small proportion of ESCC (*MLH1*, 3p22.3; 7.1%) and GC (*MSH6*, 2p16, 6.7%) without distinction among types.

Group 1: This group contains a set of genes that are perhaps the most commonly mutated genes in any human cancer. *TP53* (17p13.1) encodes an all-round tumor suppressor protein, p53, which regulates a wide network of effectors involved in the control of DNA replication, cell proliferation and survival in response to DNA damage as well as in the control of basal cell bioenergetics metabolism.[51] *PIK3CA* (3q26.3) encodes the p110 subunit of posphatidylinositol-3 kinase, an enzyme that phosphorylates phosphatidyl inositols to generate PIP3 (phosphatidylinositol 3,4,5

Table 1. Percentage of tumors with mutations in specific genes, categorized in 3 groups (see text), in UGIT cancers.[a]

Genes		OMIM #	Cytogenetic location	ESCC	EADC	GC-all	NCGC-int.	NCGC-diff.
Group 1	*TP53*	191170	17p13.1	47.4	56.3	32.2	55.6	35.1
	CDKN2a	600160	9P21	18.3	8.0	5.0	4.5	8.4
	PIK3CA	171834	3q26.3	7.8	4.5	9.6	32.6	13.3
Group 2	*NFE2L2*	600492	2q31	18.1	1.2	—	—	—
	PTCH1	601309	9q22.3	5.6	2.4	1.5	—	—
	NOTCH1[b]	190198	9q34.3	15.4	3.7	—	—	—
Group 3	*KRAS*	190070	12p12.1	0.8	4.9	6.3	9.3	4.7
	NRAS	164790	1p13.2	—	—	1.0	—	5.9
	HRAS	190020	11p15.5	0.8	O.6	2.0	6.7	1.9
	APC	611731	5q21-q22	0	5.6	6.7	10.3	2.6
	CDH1	192090	16q22.1	0	5.6	17.8	4.0	31
	CTNBB1	116806	3p22-p21.3	1.3	1.5	4.2	—	3.9

[a] = Data from COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). With the exception of NOTCH1, only genes that have been analyzed in at least 50 samples are included. Genes mutated at a frequency of > 3% are underlined.
[b] = the current dataset include only 37 tumor samples.

triphosphate), an essential second messenger in nutrient/growth factor signaling.[52] *CDKN2a* (9p21) is a complex locus that contains two overlapping open reading frames encoding $p16^{Ink4}$, an inhibitor of cell cycle progression from G1 into S phases, and $p14^{arf}$, a regulator of p53 protein stability and function.[53] Together, these 3 factors outline the "core" of cancer cellular machinery that integrates energy metabolism, growth signaling, control of replication and transcription and control of genetic stability. Alteration of these genes can be seen as having generic effects on the onset and maintenance of cancer cell phenotype, independently of tissue specificity.[54,55]

Group 2: This group may be considered as the "squamous" mutated gene group. It includes *NOTCH1* (9q34.3), encoding a member of a family of 4 transmembrane proteins receptors that regulate cell-fate during development.[56] Expression of Notch is critical for establishment and maintenance of a squamous differentiation pattern and activation of Notch-dependent pathways (also commonly observed in head and neck cancers) may represent an obligate, tissue specific event in cancers adopting a squamous architecture.[57–59] Germline mutations in *PTCH1* (9q22.3) are the underlying genetic defect in Gorlin's syndrome, a complex disease comprising multiple nevoid basal-cell epitheliomas, ovarian fibromas, medulloblastomas and skeletal abnormalities.[60,61] The *PTCH1* gene product is the receptor for Shh (Sonic Hedgehog), a secreted factor involved in the formation and differentiation of embryonic structures.[62] *NFE2L2* (also known as *NRF2*, 2q31.2) encodes a leucine-zipper transcription factor that regulates the so-called Nrf2-antioxidant response, the primary cell pathway involved in defense against the cytotoxic effect of oxidative stress. Mutations in *NFE2L2* induce loss of activation of a number of anti-oxidant, cytoprotective factors and therefore increase DNA-damage and mutagenesis caused by disrupted oxidative balance.[63] Together, *NOTCH1* and *PTCH1* underline the importance of specific morphogenetic factors in regulating the epithelial to mensenchyme interactions that lead to formation of the foregut and to squamous cell differentiation, whereas the implication of *NFE2L2* indicates the importance of inflammatory/redox deregulation among the mechanisms that drive ESCC development.

Group 3: This group somehow "mirrors" Group 2 as the "columnar" mutated gene group. It is dominated by members of the *RAS* family and by genes encoding components of the Wnt/BetaCatenin/Cadherin signaling cascade. Overall, *RAS* genes encode GDP/GTP exchange factors operating as signal transducers downstream of growth factor receptors of the tyrosine kinase family, whereas the Wnt/BetaCatenin/Cadherin pathway is the most important epithelium-to-mesenchyme signaling pathway that interconnects cell-substrate adhesion and cytoskeleton (through membrane Catenin/Cadherin complexes) with cell proliferation regulation (through cytoplasmic APC/BetaCatenin complexes and translocation of the latter to the nucleus).[64,65] Interestingly, the patterns of mutations in these genes vary by cancer topology and morphology. Mutations in *CTNNB1* are rare in EADC and are detected in about 4% of GC. Whereas *KRAS* (12p12.1) mutation seems a distinct feature in all UGIT cancers with columnar architecture, *HRAS* (11p15.5) and *NRAS* (1p13.2) mutations appear to be restricted to intestinal type and diffuse-type GC, respectively. Mutations in *CDH1* (16q22.1) and in *CTNNB1* (3p22-p21.3) appear to be distinctive features of diffuse-type GC and may contribute to define the characteristic appearance of these cancers, which consist of clusters of apparently loosely adherent cells. In contrast, mutations in *APC* (5q21-q22) are exclusively observed in intestinal type GC. It is interesting to note that, among this group of genes, the two most commonly mutated ones in intestinal-type GC are *KRAS* and *APC*, which are also commonly mutated in colorectal cancers (COSMIC database).

A recent analysis of mutation distribution in 149 EADC tumor-normal pairs has identified 26 significantly mutated

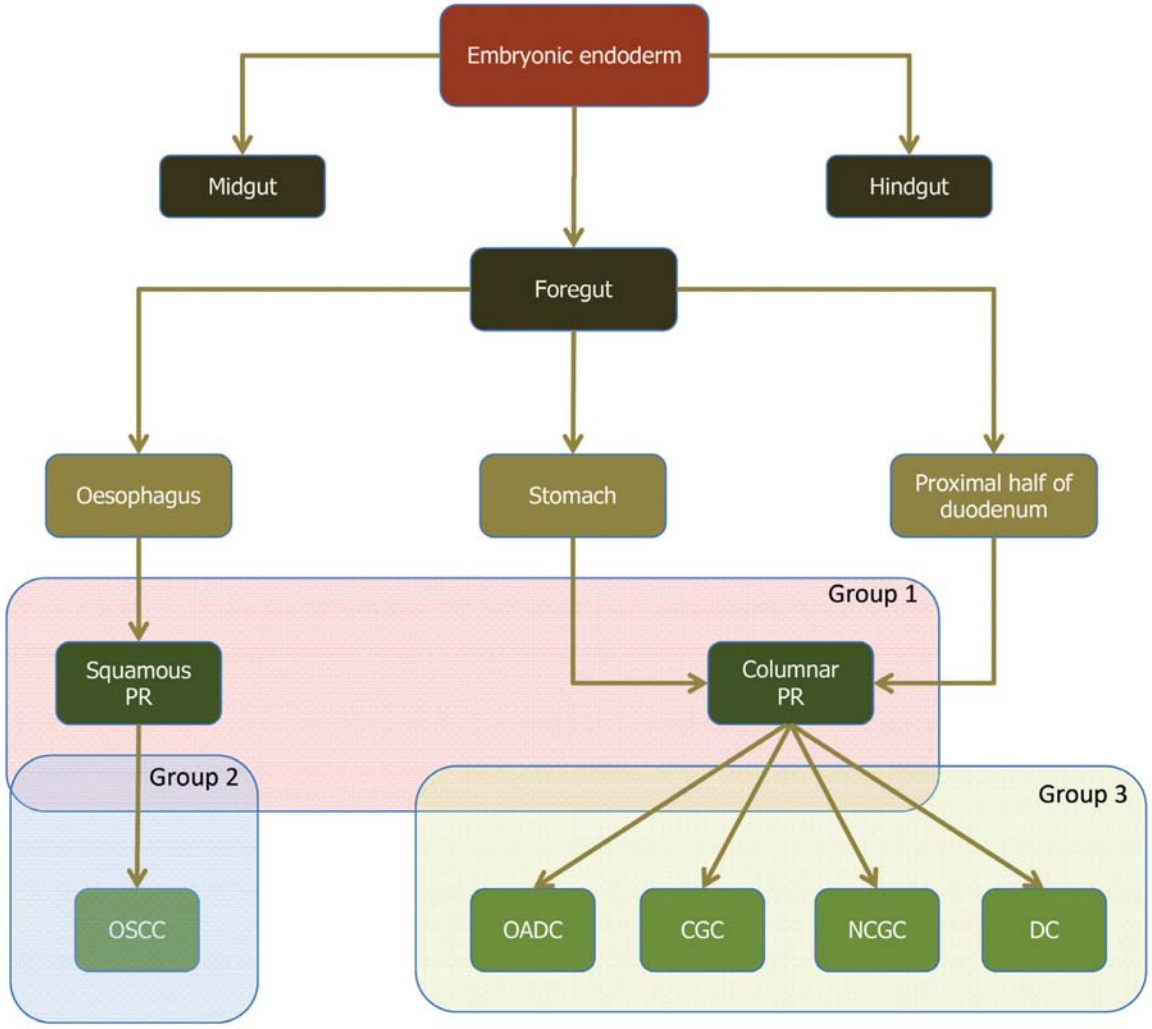

Figure 1. Embryonic differentiation and contribution of three groups of genes in the carcinogenesis of the upper gastrointestinal tract.

genes, including *TP53*, *CDKN2A* and *PIK3CA*. This study provided further evidence that other genes are common mutation targets in EADC, including *ARID1A* and *SMARC4A* (encoding the human homolog of *S. cerevisiae SNF2/SWI2* protein).[66] Interestingly, these two genes encode important regulators of chromatin remodeling that facilitates gene activation by assisting the transcription machinery to gain access to gene targets. Such mutations may contribute to an "epigenetic instability" phenotype. Data are still too scarce to determine whether such epigenetic modulators are also mutated in other types of UGIT cancers.

Conclusion: cellular and molecular phylogeny of UGIT cancers

Studies on gene mutation patterns provide interesting insights into the molecular mechanisms that drive carcinogenesis across different topographical and histological subtypes of UGIT cancers. Figure 1 illustrates how the three categories of genes discussed in this review contribute to specific pathways of histological tumor differentiation. Mutations in genes of category 1 (*TP53*, *PIK3CA*, *CDKN2A*) constitute generic alterations that activate essential functional "hallmarks of cancer", irrespective of histological type. Mutations in genes of category 2 appear to be, to a large extent, lineage specific and may occur in a selective manner in distinct types of stem cells/progenitors. Thus, tissue specificity, rather than etiology or constitutive genetic susceptibility, seems to be the key determinant for mutation patterns observed in different UGIT cancers.

In recent years, there have been wide expectations that studies on cancer-associated mutations would help identify cancer-specific targets amenable to treatment using "smart drugs" interfering with defined signaling pathways. Examples of such targets include activating mutations in genes encoding Receptor Tyrosine Kinases (RTK), which may provide a basis for pharmacological blockade using small-drug inhibitors. Current mutation patterns do not identify such types of molecular targets in UGIT cancers. In contrast, these patterns suggest that it may be possible to develop alternative therapeutic approaches by targeting the pathways of squamous cell differentiation in ESCC, and

by targeting components of the Wnt/BetaCatenin/Cadherin cascade in EADC and in GC.

Another potential clinical interest of mutation analysis is early detection of cancer using minimal samples (e.g., exfoliated mucosal cells, lavages, or circulating free DNA present in biological fluids). In the light of current data on mutation patterns, it might be feasible to design strategies based on deep sequencing of a relatively small number of genes. Indeed, presence of a potentially deleterious mutation in any of the genes listed in Table 1 in a sample of exfoliated cells or in circulating free DNA may be considered as an important criterion suggestive of the presence of an early cancer lesion. Such approaches might be of great assistance for early detection and screening in large population groups where UGIT cancers are common.

Finally, rapid accumulation of data on exome and genome-wide studies is profoundly modifying the current picture of somatic alterations in cancer. The number of genes with mutations and the number of gene rearrangements detected in any particular tumor, independently of the particular function of the genes involved, may be a better marker for malignant potential than any specific gene alteration by itself. Heterogeneous genetic patterns may be indicators of the genetic plasticity and cellular heterogeneity of tumors; two properties that support the capacity of cancer cells to rapidly evolve and adapt to escape control by microenvironment, immune response and ultimately, cancer therapy. Further studies are needed to determine whether the heterogeneity of tumor genetic pattern may be dictated by environmental factors and exposure, providing new clues for better understanding the molecular causes of cancer.

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