XVI Porto Cancer Meeting

Cancer Etiology: Bridging Worlds

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The Porto Cancer Meeting is an international annual meeting, organized by the Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), promoting the discussion of different research topics in the field of oncological diseases. This year, the IPATIMUP invited the Department of Hygiene and Epidemiology of the Faculty of Medicine of the University of Porto to co-organize its 16th edition, entitled Cancer Etiology: Bridging Worlds. The Meeting joined experts in distinct areas of the investigation on cancer etiology, and included the discussion of ongoing research projects, aiming to bring researchers from different fields together, in an attempt to encourage integrated approaches to the understanding of the causes of oncological diseases. The program illustrates some of the current challenges in the cancer etiology research, with a special focus on the need for methodologically strong studies.

Cancer mortality rates have been decreasing in many European countries over the last decade, largely due to the overall decrease in tobacco consumption, improvements in the access to screening and early diagnosis, as well as therapeutic advances. Cancer incidence and survival data further increase our ability to interpret the mortality trends, ultimately raising hypothesis for cancer etiological research.

The recognition of tobacco smoking and several infections as environmental factors responsible for a large proportion of cancers in different locations is probably the most remarkable achievement of epidemiological research on cancer etiology, but several gaps remain in our understanding of the relation between these exposures and cancer. Second-hand smoke has been classified as a human carcinogen and a cause of lung cancer for non-smokers, but results are still inconclusive for other cancer locations. The relation between *Helicobacter pylori* infection and gastric cancer is already well-established, and the hypothesis of *H. pylori* being a necessary condition for non-cardia gastric cancer remains under discussion. Despite the plausibility of the association between dietary factors and physical activity, and cancer, the progress in this field has been slower than could be anticipated three decades ago when Doll and Peto estimated that the proportion of cancer deaths attributable to diet could be as high as 70%.

These examples reflect the complex etiology of cancer and the need for wise approaches to address these topics, acknowledging the role of environmental and genetic exposures and adopting study methodologies appropriate to deal with the challenges raised by cancer etiologic research.

Meaningful advances in the understanding of the environmental causes of cancer require large studies, the study of populations with a wide variability in the exposures, the recognition of the nosological heterogeneity of cancer, accurate case definition and evaluation of exposures, and a life-course approach to disclose the causes of diseases with latency periods frequently spanning over several decades. The identification of common genetic polymorphisms associated with the occurrence of cancer has been receiving a great deal of attention in biomedical research, and the search for gene-environment interactions is frequently considered a promising field for research, but other strategies may be used to integrate epidemiological, genetic, and molecular dimensions of cancer. The principle of "Mendelian randomization" underlies the use of inherited genetic polymorphisms in instrumental variables analysis to obtain valid estimates of the association between non-genetic exposures and cancer, avoiding residual confounding and reverse causation. The understanding of the environmental causes for accumulation of genetic and epigenetic alterations in cancer may also provide valuable information to unveil its complex etiology.

All these topics were discussed in the meeting, together with ongoing research, with emphasis on national projects, namely regarding gastric, breast, colorectal and oesophageal cancers, and of a national registry of gastrointestinal stromal tumours and neuro-endrocrine tumours was presented. Summing up, we think this meeting was a great opportunity to interact personally with researchers who are at the cutting edge of cancer investigation and we gratefully acknowledge all the invited speakers, chairpersons and participants, expecting this year's Porto Cancer Meeting to have been a very pleasant event.

Cancer Mortality Trends in the European Union: Priorities for Cancer Control

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Between 1988 and 1997 age-standardized total cancer mortality rates in the European Union (EU) felt by around 9% in both sexes. Cancer mortality data in Europe up to 2002 allow a check of the forecast of further declines in cancer mortality. For men, total cancer mortality, after a peak of 191.1/100,000 in 1987 declined to 177.8 in 1997 and to 166.5 in 2002. Corresponding figures for females were 107.9/100,000, 100.5 and 95.2, corresponding to falls of 7% between 1987 and 1997, and to 5% between 1997 and 2002. Over the last five years, lung cancer declined by 1.9% per year in men, to reach 44.4/100,000, but increased by 1.7% in women, to reach 11.4%. In 2002, for the first year, lung cancer mortality in women was higher than that of intestinal cancer (11.1/100,000), and lung cancer became the second site of cancer deaths in women after breast (17.9/100,000). However, lung cancer rates in EU women still about a third of those of US women, and 50% lower than breast cancer rates. Thus, integrated and effective intervention to reduce the tobacco consumption in women should avoid the epidemic now registered in US women. Between 1997 and 2002, appreciable declines were observed in mortality for intestinal cancer in men (-1.6% per year), and in women (-2.5%), as well as for breast (-1.7% per year) and prostate cancer (-1.4%). In conclusion, there was an about 15% decline in cancer mortality for both sexes combined over the last 15 years. In absolute terms this fall corresponds to about 140,000 deaths/yr avoided (about 35,000 stomach; 40,000 intestines; 40,000 lung; 65,000 below age 65; 68,000 in men) in the EU. Thus, despite the persisting rises in female lung cancer, the recent trends in cancer mortality in the EU are encouraging and indicate that a 15% reduction in total cancer mortality between 2000 and 2015 is realistic and possible. The maintenance - and potential improvement - of favourable trends in cancer mortality in the near future requires an integrated strategy focusing on control of tobacco, alcohol abuse, and other risk factors (avoidance of obesity; physical activity; favourable changes in diet), as well as widespread adoption of advancements in cancer diagnosis and management.

Passive Smoking and Cancer

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The involuntary exposure and inhalation of environmental tobacco smoke or secondhand smoke (SHS) by non-smokers is generally referred to as passive smoking or involuntary smoking. SHS exposure is a health hazard causally associated with a number of diseases, including lung cancer, cardiovascular diseases, and acute and chronic respiratory diseases in children and adults.

With regard to cancer, the evidence is sufficient to infer a causal relationship between SHS exposure and lung cancer among lifetime nonsmokers. The pooled evidence indicates a 20 to 30 percent increase in the risk of lung cancer from SHS exposure associated with living with a smoker. There is suggestive evidence (but not sufficient) to infer a causal relationship between secondhand smoke and breast cancer. Similarly, there is suggestive evidence but not sufficient to infer a causal relationship between SHS exposure and a risk of nasal sinus cancer among nonsmokers. The evidence is inadequate to infer the presence or absence of a causal relationship between SHS and a risk of nasopharyngeal carcinoma among nonsmokers.

Along with the scientific evidence of SHS risks' on health, the public concern about SHS has increased during the last years. There are scarce standardized and comparable population-based data about SHS exposure at the European level. This is a consequence of the difficulty to measure SHS exposure. A variety of methods have been used to assess exposure to SHS. There are direct methods such as personal monitoring or biochemical measures of tobacco compounds or their metabolites obtained from body fluids, or indirect methods such as questionnaires and environmental monitoring of ambient or industrial settings. Nicotine, a highly specific marker, has also been used to identify exposure to tobacco smoke; nicotine is primarily a vapor-phase component in SHS and can be sampled using a diffusion-based nicotine monitor or an active sampler. Nicotine and cotinine can also be measured as biologic mark-

ers in the saliva, urine and blood. Epidemiologic studies assess exposure primarily by responses to questionnaires concerning the smoking habits of household members or of fellow employees. Although surveys may underestimate real exposure, they are helpful to obtain information about the characteristics of the exposure and the exposed people, including other relevant information to characterize SHS exposure.

Nutrition, Physical Activity and Human Cancer: Advantages and Weaknesses of Epidemiological Study Designs

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Over the last 50 years, the epidemiological research on diet and cancer matured. Hundreds of case-control and cohort studies have been conducted this far and several large human intervention trials have been undertaken. The results of these studies are mixed. While a high intake of dietary fat appeared to increase breast cancer risk in etiological and case-control studies, we now conclude from the results of cohort and intervention studies that it may not importantly contribute. We have become aware of the fact that for each descriptive and analytical epidemiological study design advantages and potential weaknesses can be identified. The most common traps in epidemiological research concern cause-effect relationships and issues of confounding. Also, information and selection bias may distort our conclusions, as well as pitfalls of dietary assessment methods. The absence of convenient intermediate biomarkers of disease, such as cholesterol in cardiovascular diseases, remains a challenge in cancer epidemiology.

In the future, large cohort studies with a wide variation in dietary habits using biomarkers of dietary intake, while taking genetic variation into account, may enable us to show stronger association between diet and cancer.

In this presentation, the reasons for the complexity of investigations of the nutritional epidemiology of cancer will be considered.

Is *Helicobacter pylori* Infection a Necessary Condition for Non-cardia Gastric Cancer? A View from Epidemiology

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Current knowledge implies that acquisition of *Helicobater pylori* seems to occur predominantly in childhood and that once acquired the infection persists a life long in most infected subjects. Infection with *H. pylori* is associated with a chronic gastritis in children as well as in adults. *H. pylori* infection plays a causal role in the development of duodenal and gastric ulcers and should be eradicated in case of diagnosis of such a condition. It has been suggested that up to 95% of duodenal and 70% of gastric ulcers are attributable to this infection and most cases occur in middle aged subjects. Also, numerous studies have reported an increased risk of gastric cancer, in particular non-cardia gastric cancer, among subjects infected with *H. pylori*. It was further suggested that gastric cancer is more strongly linked with *H. pylori* strains that have the cytotoxin-associated gene (*cagA*) which encodes a highly immunogenic high-molecular (120 kd) protein (CagA) than with other strains. The meaning of this virulence marker may vary geographically. The epidemiologic evidence relating *H. pylori* infection with gastric cancer came initially from 3 nested-case control studies which all showed that cancer patients had a higher *H. pylori* seroprevalence compared to controls and the risk associated with a positive serology varied between 2.1 and 8.7. A meta-analysis of 19 cohort studies and case-control studies published estimated a summary odds ratio (OR) of 1.92, indicating an approximately a 2-fold risk of gastric cancer among infected individuals. Several others came to a similar result indicating a relative risk between two and three. It has been suggested, however, that associations might have been underestimated due to clearance of the infection in the course of disease development and progression in some of the cases with gastric cancer. We

will discuss findings that support suggestions that the *H. pylori* - gastric cancer relationship is much stronger than previously thought, and they suggest that *H. pylori* infection may even be a necessary condition for development of non-cardia gastric cancer.

Accumulation of Genetic and Epigenetic Alterations: A Key Causal Process Between the Environment and the Etiology of Cancer

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Accumulation of genetic and epigenetic alterations is often a fundamental etiopathogenic process in diseases of complex etiology. And it often has environmental causes. The diseases most prevalent in postindustrial societies are caused (biologically) by survival, growth and selection of cell clones that have accumulated multiple genetic and epigenetic alterations. Technology-driven research ('omics-research' or otherwise) should not make us forget that there often is a well established causal relationship between certain environmental factors, acquired alterations and clinically relevant phenotypes; e.g., in cancer, somatic mutations in the *K-ras*, *p53* and other genes. Disregard for the environmental causes of the accumulation of genetic and epigenetic alterations in diseases of complex etiology is one of the features ideologically more characteristic, socially more relevant and, nonetheless, with a weaker scientific basis of contemporary biomedical research. Epigenetics is the study of changes in gene expression that are not regulated by what traditionally has been viewed as the DNA nucleotide sequence; e.g., gene silencing by promoter hypermethylation or histone modification. There currently is a re-emergence of knowledge on the influence of environmental agents on gene expression; e.g., the carcinogenicity of compounds like nickel, cadmium or arsenic involves DNA hypo- and hypermethylation and histone deacetylation, which contribute to heterochromatin condensation and the epigenetic silencing of some genes (Luch A, Nature Reviews Cancer 2005; Jablonka E, Int J Epidemiol 2004; Porta M & Crous M, Gac Sanit 2005). A fraction of biomedical research is centred on genetic variants inherited and of low penetrance; e.g., on genes that are said to confer individual 'susceptibility'. Fortunately, another part of biomedical research deals with: a) the population impact of reducing environmental exposures; b) the causes of acquired genetic alterations; or, as part of the latter, on b1) environmental exposures as causes of acquired genetic and epigenetic alterations. It is scientifically wrong to consider 'genetic' as synonymous of 'inherited', and viceversa: somatic (acquired) genetic alterations and sociocultural inheritance cannot be ignored when research aims at improving the primary prevention of cancer.

Mendelian Randomization: Understanding the Environmental Causes of Cancer

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Observational epidemiology has produced many spurious findings owing to uncontrolled confounding and reverse causality among other reasons. Measurement of many potentially interesting environmental exposures is difficult because they are either intrinsically difficult to measure - for example, dietary intake, or because they are very strongly socially patterned. Consequently, understanding of the causal nature of observed associations is frequently not sufficiently trustworthy to use for health policy or prevention practice.

Mendelian randomization, the use of common genetic polymorphisms as surrogates for measuring exposure levels in epidemiologic studies, provides one method of assessing the causal nature of some environmental exposures. This can be illustrated by looking at the association between the *ALDH2* polymorphism and esophageal cancer. Alcohol drinking is considered a risk factor for esophageal cancer, and exposure to high levels of acetaldehyde, the principal

metabolite of alcohol, may be responsible for the increased cancer risk. The ability to metabolize acetaldehyde is encoded by the *ALDH2* gene, which is polymorphic in some populations. The *ALDH2*2* allele produces an inactive protein subunit, which is unable to metabolize acetaldehyde. An individual's genotype at this locus may influence their esophageal cancer risk through two mechanisms, first through influencing alcohol intake and second through influencing acetaldehyde levels. It has been shown that esophageal cancer risk is reduced among *ALDH2 *2*2* homozygotes and increased among heterozygotes relative to *1*1* homozygotes. This suggests that alcohol intake increases the risk of esophageal cancer and that acetaldehyde plays a carcinogenic role in esophageal cancer.

Other potentially relevant examples are the use of genetically determined variation (e.g. the *TAS2R38* haplotype) in the perception of the bitter taste of cruciferous vegetables which may be implicated in colonic and other cancers.

Mendelian randomization, in common with all genetic epidemiology studies, requires large sample sizes to have sufficient power to detect relevant associations, and good understanding of functional genomics and the regulation of the relevant metabolic pathways.

Mucin Glycosylation of the Gastric Mucosa and *Helicobacter pylori* Infection

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Evidence from experimental studies demonstrate that both the protein part and the carbohydrate component of gastric mucins are fundamental for the establishment of *Helicobacter pylori* infection of the gastric mucosa. Correspondent studies in human tissues have produced conflicting results that need further clarification.

We will present data that contribute to understand the role played by the gastric mucin MUC5AC and of the intestinal mucin MUC2 for adhesion and clearance, respectively, of *Helicobacter pylori*. We will also address the molecular mechanisms, namely CDX2 intestinal homeobox gene expression, that control mucin gene expression. The role played by the carbohydrate components of human mucins will also be presented, particularly the relative importance of the H type 1 fucosylated structure and of Lewis b difucosylated structure for adhesion/infection by BabA positive *Helicobacter pylori* strains. Recent observations by our group suggest the preponderance of H type 1 structure (that defines the Secretor status) over the expression of Lewis b. Expression of sialyl-Tn, synthesized by ST6GalNAc-I, and of α1,4-GlcNAc in clearance of *Helicobacter pylori* will also be discussed. Finally, we will present data that relate MUC1 gene VNTR polymorphism to *Helicobacter pylori* adhesion and preliminary data suggesting that glycosyltransferases can be induced in gastric cells by co-culture with *Helicobacter pylori*, thus contributing to explain glycosylation changes induced by gastric mucosa infection.

Gastric Lesions in Portugal and Mozambique: The African Enigma

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Gastric cancer is one of the most frequent cancers, both in developed and in developing countries, with a wide variation in incidence and mortality rates across geographical areas. *Helicobacter pylori* is an established major risk factor for gastric cancer, to which approximately two thirds of all cases are attributed, but only a small proportion of the infected subjects develop cancer. The incidence of gastric cancer varies widely across regions with high prevalence of infection, and is unexpectedly low in many African and Asian countries with a large proportion of the adult population infected, depicting the so called African and Asian "enigmas". Also, intestinal metaplasia is relatively infrequent in *H. pylori*-infected subjects from African and South-Eastern Asian countries, extending the concept of the "enigmas" to precancerous lesions. Differences in the virulence of the infecting strains, the host genetic background and other environmental exposures are plausible explanations for the variation in gastric cancer rates in countries with similar *H. pylori* prevalence.

Our approach to this topic started with ecological studies that support the hypothesis of a synergistic effect of *H. pylori* and smoking for the occurrence of intestinal metaplasia and gastric cancer, suggesting that the low cigarette consumption found in most African and South-Eastern Asian countries contributes to the low gastric cancer frequency in these regions, despite the high prevalence of infection. Currently we are addressing the association between environmental and genetic exposures and the occurrence of intestinal metaplasia in Portugal and Mozambique, taking advantage of the differences in sociodemographic and behavioural factors between these populations, and the characterization of outcomes and exposures using standardized methodology in both investigations.

The African setting may provide valuable clues for the understanding of the "enigmas", and the factors that modulate carcinogenesis when *H. pylori* infection is present. Improvements in the knowledge of cancer patterns in Africa, and well-designed prospective research, namely following pre cancerous lesions, are needed.

Establishing Two Disease Specific Tumours Registries: ReGIST and ReGENE

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Gastrointestinal Stromal Tumours (GISTs) and Neuro-Endocrine Tumours (NETs) have a low incidence that is not well characterized in the Portuguese population. The establishment of a national informatics registry of clinical parameters of pathologically and molecular validated GISTs (ReGIST) and NETs (ReGENE) will help to create a Tissue Tumour Bank on these tumours at IPATIMUP. The legal and ethical aspects for the implementation of this project were approved recently by the National Commission for the Protection of Data (NCPD).

The by-laws of the ReGIST and ReGENE were previously approved by representative members of the multidisciplinary specialities involved in the diagnosis, treatment and study of GISTs and NETs after several open meetings with ample discussion.

The accrual of cases will be based on the parameters of several check- lists included in an encrypted web-site of IPATIMUP with back-up system for logins. Individual password access will be restricted to medical doctors regularly registered in the Portuguese Medical Association (users). The validation of cases will be based on the (histological, immunohistochemical and molecular assessment, whenever appropriate) evaluation of tumour tissue samples (e.g., paraffin-blocks) and the corresponding form signed by the user, after reception at IPATIMUP in a specially created envelope. The Pathology Laboratory of IPATIMUP has the accreditation certificate of the College of American Pathologists.

Patient informed consent will be compulsory for prospective cases and obtained by the users. The users may share and follow-up their cases and validation results with other users, complying with the management of medical data as defined by the Deontological Portuguese Medical and the NCPD rules.

The general results as well as the audit of the quality of the registries/tumour tissue banks will be periodically analyzed and publicized.

Our main goals with this project are: a) to stimulate multidisciplinary scientific studies on the biopathological features and therapeutical targets in patients with GISTs and NETs; b) to clarify still controversial aspects (e.g. primary/acquired resistance to therapy) that may help define potential best tailored management of patients harbouring these uncommon type of tumours.

Molecular Epidemiology of Hereditary Breast Cancer in Portugal

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Until very recently, the spectrum of mutations in *BRCA/2* genes in the Portuguese population was largely unknown. Although founder *BRCA1/2* mutations have been described in several populations, that was an unlikely finding in our Portugal due to our heterogeneous background.

Our studies started with the general analysis of these genes, in high risk breast/ovarian families selected using strict criteria. Of the recurrent mutations observed in our sample a rare *BRCA2* rearrangement was a candidate for a founder mutation and haplotype studies were undertaken. Screening of all high-risk consecutive families, counselled in our practise for this genetic event revealed a total of 20 non-related positive families. Positive individuals shared a common haplotype estimated to have occurred 2400-2600 years ago. This demonstrates the founder effect of this *BRCA2* rearrangement and has implications in the genetic screening of *BRCA1/2* gene mutations in breast/ovarian families of Portuguese ancestry. Further studies have allowed us to classify different phenotypes associated with this mutation and several phenotypic details like the incidence of male and prostate cancer associated with this genetic event are being investigated.

It's interesting to note that, besides the founder effect of the rearrangement described; several *BRCA1* and *BRCA2* mutations are recurrent in our population. A collaborative effort is under way to investigate the geography origin of families sharing the same mutations.

Apoptosis and Inflammatory Response in Colorectal Carcinogenesis: Interaction Between the *p53*, *ß-catenin* and *TGF-ß* Pathways in the Prognosis and Resistance to Therapeutics

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Colorectal cancer (CRC) is the first cancer-related cause of death in Portugal. Although surgery alone is potentially curative in early stage CRC, many patients present to the clinic with node-positive CRC, a stage warranting adjuvant 5-Fluorouracil (5-FU)-chemotherapy to decrease the risk of recurrence. 5-FU is a thymidylate synthase inhibitor that incorporates FdUTP in DNA, inducing cell cycle arrest and apoptosis. Resistance to 5-FU chemotherapy is known to be a major cause for treatment failure though the clinical-biological features that may help predicting it are largely unknown. Right and left-sided CRC usually have distinct histological characteristics which seem to result from diverse genetic alterations, namely the presence of microsatellite instability (MSI) due to deficiencies in the DNA mismatch repair system, which are more frequent on right-sided CRC. A better prognosis has been associated with these right-sided MSI+ tumors that share certain histological features like intra-tumoral lymphocytes (presumably a host inflammatory response to the tumor) and a high frequency of somatic mutations in genes with highly repetitive sequences like β-*catenin/TCF-4* (β-*catenin/TCF-4* pathway), *TGF(RII* (*TGF*β *pathway*) and *BAX* (*p53* pathway). On the other hand left-sided CRC characterized by chromosomal instability frequently show no MSI, are associated with a poorer prognosis and often have mutations on tumor suppressor genes like *APC* (β-*catenin/TCF-4 pathway*), *SMAD4* (*TGF--ß pathway*) or *TP53* (*p53* pathway). This genetic heterogeneity may explain conflicting results regarding prognosis in recent reports and it is conceivable that different responses to 5-FU in CRC with different MSI status reflect intra- and/or inter-tumor heterogeneity as which genes are affected. To investigate this possibility we propose to analyze, in a series of advanced stage CRC patients previously characterized as to their MSI status, molecular alterations of the β-*catenin/TCF-4*, *TGF*β and *p53* pathways which are known to be simultaneously related to colorectal carcinogenesis and signaling pathways that regulate apoptosis and inflammatory responses.

The findings will be subsequently correlated with the clinical response to 5-FU-based regimens in an attempt to identify new predictive markers of 5-FU-response in patients with CRC.

Study of the Gastric and Intestinal Differentiation in Barrett's Esophagus

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Barrett's esophagus is a premalignant condition in which the normal squamous epithelium of the esophagus is replaced by an abnormal columnar lining. It arises as a consequence of chronic gastro-esophageal reflux and represents

the only recognized precursor of esophageal adenocarcinoma. Although the diagnosis of Barrett's esophagus requires the presence of intestinal metaplasia with goblet cells, the metaplastic epithelium is a complex and heterogeneous admixture with gastric and intestinal features.

The intestinal phenotype has been most extensively studied due to its association with cancer risk. It is characterized not only by the presence of goblet cells, required for the diagnosis of Barrett's esophagus, but also by columnar non-goblet cells. Goblet cells typically express intestinal type mucin MUC2. Previous reports from our group demonstrated that the columnar non-goblet cells also express markers of intestinal differentiation such as the brush border enzyme sucrase-isomaltase (SI).

The gastric phenotype has been less studied but our previous work demonstrated the wide expression of gastric type mucins (MUC5AC and MUC6) by columnar cells of gastric and intestinal type epithelium as well as by goblet cells of intestinalized areas.

Our research project aims at evaluating the factors involved in the development and maintenance of the Barrett's metaplastic phenotype.

We hypothesized that the same factors involved in the development of gastric and intestinal epithelia during embryogenesis might also be involved in the development of the metaplastic phenotype of Barrett esophagus. Accordingly, we propose to study the correlation between the expression of markers of intestinal (MUC2 and SI) and gastric (MUC5AC and MUC6) differentiation and the transcription factors responsible for the development of normal intestinal and gastric epithelia during embryonic development (CDX2, PDX1 and SOX2).

We are also interested in studying the mechanisms responsible for the abnormal expression of genes codifying intestinal and gastric proteins in Barrett's esophagus. One candidate mechanism is DNA methylation, a process of epigenetic regulation of gene expression which has recently been demonstrated to be involved in tissue-specific expression of several gene products. Mucin gene regulation by DNA methylation has been demonstrated in cancer but it is still unknown whether it might also operate in non-neoplastic tissues. On the other hand, abnormalities in the methylation patterns of several genes (such as *p16* and *APC*) have been described in non-neoplastic Barrett's esophagus. We pretend to evaluate whether abnormal methylation of the promoter regions of the mucin codifying genes *MUC2*, *MUC5AC* and *MUC6* is involved in the aberrant expression of this proteins in Barrett's esophagus.

Case Definition in Cancer Etiologic Research: Surrogate Endpoints, Nosological Heterogeneity and Quality Assessment

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This presentation will focus on gastric cancer as a model for cancer etiology research and for the discussion of nosological heterogeneity, surrogate endpoints and quality assessment.

Gastric cancer is very heterogeneous from the morphologic standpoint. This heterogeneity is amply reflected in the diversity of histopathologic classifications on record which are based on different approaches: histologic profile, degree of differentiation, pattern of growth and histogenesis. The classification of Laurén is one of the most widely used, recognizing two major types of gastric cancer: "intestinal" carcinoma and "diffuse" carcinoma, which display different clinicopathologic profiles and occur in distinct epidemiologic settings.

The large majority of gastric cancers are sporadic. However, familial aggregation of gastric cancer, both of the diffuse and the intestinal type, suggest the importance of genetic predispositin in these settings. Presently, it is calculated that about 1% of gastric cancers are hereditary, the majority corresponding to "Hereditary Diffuse Gastric Cancer" (HDGC), caused by germline mutations of E-cadherin gene (*CDH1*).

Risk factors for the development of sporadic gastric cancer encompass environmental factors (diet, *Helicobacter pylori* infection, etc) and host factors. Both *Helicobacter pylori* genotype (regarding *vacA* and *cagA* genes) and host genotype (pro-inflammatory host genetic polymorphisms in *IL1B*, *IL1RN* and *TNFA* genes and *MUC1* gene polymorphism) influence the risk of gastric cancer development.

In sporadic gastric cancer, the available evidence supports the existence of two main histogenetic pathways of carcinogenesis: one leading to "intestinal" carcinoma via chronic atrophic gastritis, incomplete intestinal metaplasia, namely type III and adenomatous dysplasia, and the other leading to diffuse carcinoma, either *de novo* or via hyper-

plastic changes. Both pathways appear to develop on the background of *Helicobacter pylori* associated gastritis.

The discussion of surrogate endpoints will take into consideration studies performed in Northern Portugal for the identification of precursor lesions of gastric cancer and the "Intestinal Metaplasia Intervention Study" conducted by the European Cancer Prevention Organization (ECP) in 9 European countries.

The issue of quality assessment will take into consideration the experience obtained in the frame of EPIC/EUR-GAST study on "Pathology findings and validation of gastric and esophageal cancer cases in a European cohort". This study described the incident gastric and esophageal cancer cases identified within the European Prospective Investigation into Cancer and Nutrition (EPIC), a large prospective European cohort. A nested case-control study was developed within this cohort (EUR-GAST), focused on the analysis of risk factors for gastric and esophageal cancer development, which required the classification of all cancer cases (histotype and localization). This study confirmed the relevance of validation studies, notably for multicenter studies.

A Life Course Approach to Cancer Aetiology: Exploring Birth Cohort Studies

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Cancer is expected to occur as the result of subtle changes that may take several years to settle, sometimes a lifetime. There must be acute events at critical periods or chronic additions of exposures or both. Though genetics are expected to change over time (probably), environmental factors are major contributors to disease occurrence by acting at different sites and moments during carcinogenesis. Cohort studies allow an accurate assessment of the longitudinal course of exposure and the investigation of multiple outcomes. The opportunities to produce meaningful evidence on the causes of cancer in a life-course perspective are larger if the participants are enrolled in the cohort at younger ages, but the resources and logistic difficulties to undertake this type of research increase substantially.

This presentation focus on Public Health, presented as a framework for research and for action. The use of epidemiologic tools to answer questions about diseases of complex aetiologies is presented as a comprehensive approach that takes on board other basic sciences both from the biological world as the social one. The fundamental difference between cause or causal mechanism (a complex of additive triggers) and circumstances of disease occurrence is firmly emphasised. Cohorts require a long time to complete and a large amount of resources, human, technical and financial, presenting challenge in different ways and Public Health, "ultimately a question of what kind of society we wish to live in" (*Sweden 2004, National Health Plan*), is at the core of this type of research.

The Role of Large Collaborative Studies in the Unraveling of Cancer Determinants: The EPIC Cohort

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Introduction: It is widely accepted that nutrition has an important role in cancer occurrence. However, in spite of decades of epidemiological investigation, scientific evidence on several cancer sites is still insufficient and prevent the establishment of solid conclusion. The European Prospective Investigation into Cancer and Nutrition (EPIC) was specifically designed to investigate the relationship between diet and cancer, with the aims of making a significant contribution to the accumulated scientific knowledge, trying to overcoming limitations of previous study. We present the most relevant results obtained so far for the most frequent cancer sites.

Methods: EPIC is a multi-centre prospective study carried-out in 23 centres from 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom, including 521,468 subjects (368,010 women and 153,447 men), most aged 35-70 years.

Results: Consumption of fruit is negatively associated with cancer of the lung and cardia gastric cancer, but is not

associated with prostate cancer and breast cancer. Consumption of vegetables, mainly onion and garlic, probably reduces the risk of the intestinal stomach cancer but probably is not associated with cancer of the lung, prostate and breast cancer. Consumption of red and processed meat is positively associated with colorectal cancer and with non-cardia stomach cancer. Fish and fibre intake are negatively associated with colorectal cancer risk. High alcohol intake increases the risk of breast cancer.

Conclusion: These first results from the EPIC study on main food groups and most frequent tumours have made a significant contribution to the already accumulated evidence, and in combination with data from other prospective studies, as well as from studies using biological markers, provide the scientific knowledge for appropriate public health strategies aimed at reducing the global cancer burden.