Hepatocarcinogenesis: chemical models
Introduction

- Earliest observations that human exposure to certain chemicals is related to an increased incidence of cancer
- John Hill 1761
  - Nasal cancer in snuff users
- Sir Percival Pott 1775
  - Scrotal cancer in chimney sweeps
  - Soot and coal tar
Experimental chemical carcinogenesis

- Yamagiwa and Ichikawa 1918
- Multiple applications of coal tar to rabbit ears produced skin carcinomas
- First demonstration that a chemical could produce cancer in an animal
- Confirmed Pott’s initial observation and linked human epidemiology and animal carcinogenicity
Somatic mutation theory

- Theodor Boveri 1914
- Concept that cancer involves an alteration in the genetic material of the somatic cell
  - Chromosome abnormalities
- Furth and Kahn 1934
- Isolated single cell clones from a tumor and showed that injection into a healthy host could reproduce disease
  - Cancer = stable heritable cellular alteration
Chemical carcinogenesis

- James and Elizabeth Miller 1950s
- Observed that a wide variety of structurally diverse chemicals could produce cancer in animals
- Proposed that all of these chemicals require metabolic activation to electrophilic reactive intermediates
  - Covalently bind to nucleophilic centers on proteins, RNA, or DNA
  - Electrophilic theory of chemical carcinogenesis
Evidence for genetic mechanisms

1) **Cancer is a heritable stable change**
2) **Tumors are generally clonal in nature**
3) **Many carcinogens are metabolized to electrophilic intermediates that covalently bind to DNA**
4) **Many carcinogens are also mutagens**
5) **Many cancers display chromosomal abnormalities**
6) **Transformed phenotype can be transferred from a tumor cell to a non-tumor cell by DNA transfection**
Genotoxic agents

- Direct acting carcinogens
  - $N$-methyl-$N$-nitrosourea (MNU)
  - $N$-methyl-$N'$-nitro-$N$-nitrosoguanidine (MNNG)
- Indirect acting carcinogens
  - DimethylNitrosamine (DMN)
  - Benzo[a]pyrene
- Radiation
- Inorganic agents
Epigenetic agents

• Immunosuppressive xenobiotics
• Asbestos
• Hormones
• Promoters
  - 12-O-tetradecanoylphorbol-13-acetate
  - Phenobarbital
Evidence for epigenetic mechanisms

1) Cancer is associated with altered differentiation and proliferation
2) The cancerous state of tumors is sometimes reversible
3) Carcinogenesis is induced by non-mutagenic substances
4) Not all carcinogens are mutagens
5) Carcinogenesis is associated with changes in DNA methylation
Multistage carcinogenesis

• Initiation
  - Genotoxic event
• Promotion
  - Clonal expansion of an initiated cell
• Progression
  - Development of a malignant tumor
Initiation-promotion model

• 12-O-tetradecanoylphorbol-13-acetate (TPA) belongs to a family of compounds called phorbol esters that are isolated from croton oil and are active almost exclusively on mouse skin
• TPA is also known as phorbol 12-myristate 13-acetate (PMA)
• Phenobarbital, DDT, chlordane and TCDD are hepatic tumor promoters
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Features of tumor promoters

1) Following a sub-threshold dose of an initiating carcinogen, chronic treatment with a tumor promoter will produce many tumors

2) Initiation at a sub-threshold does alone will produce very few if any tumors

3) Chronic treatment with a tumor promoter in the absence of initiation will produce very few if any tumors

4) The order of treatment is critical: initiation must precede promotion
Mouse skin model

• Berenblum 1941
  - Alternating doses of croton oil and benzo[a]pyrene

• Mottram et al.
  - Single sub-effective dose of benzo[a]pyrene followed by repetitive croton oil treatments
    1) SW mice 200 nmol DMBA
    2) 1 week later, 2-5 nmol TPA twice a week for 20 weeks
    3) After 15 weeks, 12-14 benign papillomas
Mechanisms of tumor promotion

- Clonal expansion of initiated cells by providing a selective growth advantage, or by repressing normal cell growth, or both
- The specific phorbol ester is protein kinase C (PKC)
  - Serine and threonine kinase and a $Ca^{2+}$ and phospholipid-dependent enzyme
  - Diacylglycerol is also a potent tumor promoter in mouse skin
Rodent models of liver cancer

- Most rat strains have < 5% lifetime incidence of primary hepatocellular tumors
- In contrast, outbred Swiss Webster mice have 35% incidence in males and 5% incidence in females
- In the B6C3F1 (National Toxicology Program; NTP) mouse the range is 25-40% for males and 4.6-9.7% for females
- In bioassays for carcinogenicity, the liver is the most commonly affected site
Hepatic carcinogenesis

- 2 major pathways have been described
  - Oval cell proliferation leading to lesions composed of extensive connective tissue matrix investing a metaplastic ductal system (cholangiofibrosis or adenofibrosis)
  - Altered hepatic foci, hepatic nodules, and hepatocellular carcinoma (HCC)
- Much of our current understanding comes from nitrosamine or aflatoxin studies in rats (relatively non-toxic at carcinogenic doses)
Altered hepatic foci

- Hepatocellular tumors develop from foci of altered hepatocytes
- Increased eosinophilia, or basophilia, or because of rearrangement of RER, may be striped or tigroid in appearance
- In the rat, many foci express fetal enzymes such as gamma-glutamyl transferase (GGT) and the placental form of GSH S-transferase
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Some aspects poorly understood

- The changes are not seen in all foci
- Foci in mice do not have GGT or placental GSH S-transferase
- Whether all foci develop into tumors is not known
- The origin of the foci is also not known
- As they grow, the foci become nodules
2-step hepatocarcinogenesis

• Initiation followed by promotion
• Rodents appear to have no absolute requirement for deliberate exposure to genotoxic carcinogens for neoplasia to develop
  - Spontaneously initiated cells in the liver
  - Low-level environmental exposure to genotoxic carcinogens or inherent metabolic processes leading to oxidative stress?
Genotoxic hepatocarcinogens

- Metabolic activation of dimethylnitrosamine (DMN) or diethylnitrosamine (DEN)
- Ultimate carcinogen is methyl diazonium ion
- Methyl carbonium ion forms pre-mutagenic $O^6$-guanine and $O^4$-thymidine
Epigenetic hepatocarcinogens

• 2 classes have been widely investigated
  - Phenobarbital (PB)
  - Peroxisome proliferators
• PB causes induction of mixed function oxidase enzymes
• Causes liver enlargement as well as CYP enzyme induction
  - Hyperplasia, hypertrophy of cells in centrilobular region (due to proliferation of SER)
PB promotion

- If PB is given to rats for $\geq 18$ months, there may be a small increase in the number of hepatic tumors.
- If treatment is preceded by short exposure to genotoxic carcinogen such as DEN, administration of PB results in considerable tumor burden.
- With PB treatment, foci have up to 10-fold increase in mitotic activity and decreased apoptosis.
Peroxisome proliferators

- Chemically heterogeneous group
- Phthalate esters most widely studied
  - Hypolipidemic agents based on clofibric acid, or unrelated tibric acid, and WY-14643
- Mice and rats > hamsters > guinea pig > primates
- Hyperplasia and cellular hypertrophy with massive expansion in size and number of peroxisomes (approximately 10-fold increase)
- Cytoplasmic receptors belong to steroid hormone receptor superfamily are peroxisome proliferator activated receptors (PPARs)
Peroxisome induced tumors

- Chronic administration of agents that induce peroxisome proliferation results in accumulation of lipofuscin in the liver and development of HCC in mice and rats
- Basophilic foci give rise to basophilic nodules, then to trabecular carcinomas
- Different from spontaneous foci in the rat
  - Negative for GGT and placental GSH S-transferase
- Hyperplasia plus oxidative stress
Helicobacter-Associated Hepatitis and Hepatocellular Neoplasms in Control A/JCr Male Mice

Fox et al. 1994
Ward et al. 1994
*H. hepaticus* in A/J mouse liver and colon

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Similar Paradigm for *Helicobacter hepaticus*
Progression of Pre-Malignant Liver Changes

Lobular Hepatitis  Dysplasia  Hepatocellular carcinoma

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