UPDATE ON LYMPHOMA PATHOLOGY: II THE ROLE OF GENETIC MUTATIONS IN THE PATHOGENESIS OF LYMPHOID NEOPLASIA

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INTRODUCTION

In a sense we know the cause of cancer; it is genetic mutations^(1,2). These operate by progressively damaging the mechanisms for control of the cell mitotic cycle. For mutations to do this several conditions must apply:

- the mutations must somehow escape the cell's DNA repair mechanisms
- the mutant cell must be able to enter the mitotic cycle by evading normal mechanisms which prevent this
- the mutant cell must survive the effects of the mutation and be able to pass on its defective DNA to succeeding generations of its progeny.

When sufficient mutations have accumulated in its mitotic control genes, a cell can break free of mitotic control and become a cancer cell.

MUTATIONS

(a) What is a mutation?⁽³⁾

Human body cells, apart from red blood cells and germ cells, contain in their nuclei 50,000 genes encoded within DNA molecules comprising 6×10^9 base pairs, and packaged into two sets of 23 chromosomes. A mutation is a change in the DNA molecule, and we refer in this paper to permanent changes, uncorrected by DNA repair mechanisms. Mutations range from a change in a single base-pair of DNA to loss or duplication of an entire chromosome (Figure 1).

Translocations are found particularly commonly in lymphoid neoplasms⁽¹⁾ (Figure 2).

Figure 2 shows the common translocation in Burkitt's lymphoma in which the growth promoting gene *c-myc* is

CHROMOSOME NUMBER

Triploidy (3N)* in 2% spontaneous abortions
Aneuploidy eg Down's Syndrome (N = 47)

STRUCTURAL CHROMOSOME ABNORMALITIES

Translocations Swap of segment of DNA between chromosomes
Deletions loss of a segment of DNA
Duplications doubled segment of DNA
Inversion DNA segment reversed after repair

POINT MUTATION

Change of a single DNA base-pair

Figure 1 Common types of genetic mutation (*N = number of chromosomes)

placed next to the immunoglobulin heavy chain gene on chromosome 14 and thereby activated.

(b) Causes of mutation

The vast majority of mutations can be regarded as rare random errors in the complicated process of DNA replication, with a minor contribution from background radiation, chemicals, and free radicals. Most mutations, therefore, occur spontaneously during mitoses or meiosis. Conditions of chronic lymphocyte proliferation such as *Helicobacter* infection of the stomach, or Hashimoto's thyroiditis are associated with an increased incidence of lymphoma.

(c) Rate of mutation

The "normal" mutation rate in human cells is difficult to estimate, but evidence from the incidence of new cases of dominant mutations (such as Huntington's Chorea), *in vitro* cell culture, and studies on the rare eye tumour, retinoblastoma, suggest a figure of about one mutation per gene per mitosis per 10 million cells⁽⁴⁾. Is that enough to cause cancer? We shall shortly see that it is. Furthermore there is evidence

Figure 2 Translocation of *c-myc* gene from chromosome 8 to 14

that the mutation rate is not constant but increases with age, perhaps exponentially⁽⁵⁾.

(d) Consequences of mutation

Whatever the cause of the mutation, the result with respect to a particular gene will be one of the following:

- no effect
- inactivation of the gene
- inappropriate activation of the gene.

Inappropriate activation of a gene is frequently seen as a result of translocations in lymphoid neoplasia.

(e) How many mutations are needed to produce a cancer?

Most human cancers contain multiple mutations. The number of mutations and the genes responsible are under intensive investigation, but analysis of age-specific incidence curves (see Part I) has suggested as many as seven mutations, and certainly more than one⁽⁶⁾.

(f) A mathematical digression into acute lymphoblastic leukaemia

One can get quite a good idea of the number of mutations required to produce acute lymphoblastic leukaemia by the study of data (Figure 3). The incidence of acute lymphoblastic leukaemia is 1/100,000/year and it is estimated that there are 10¹³ lymphocyte divisions per year in a child⁽⁷⁾. Mutation rate per gene per cell division is about 10⁻⁷.

The probability of two independent events each of probability p is p x p, and of n independent events, pⁿ.

Mutation at n specified sites	Probability (risk) per year per person	Predicted Occurrence per 100,000 people ie. incidence rate
n = 1	10 ¹³ x 10 ⁻⁷ = 10 ⁶	10 ¹¹
n = 2	10 ¹³ x (10 ⁻⁷) ² = 10 ⁻¹	10,000
n = 3	10 ¹³ x (10 ⁻⁷) ³ = 10 ⁻⁸	0.001
n = 4	10 ¹³ x (10 ⁻⁷) ⁴ = 10 ⁻¹⁵	10 ⁻¹⁰

Figure 3 Predicted incidence rates of acute lymphoblastic leukaemia from models using one to four mutations

(g) Conclusions from mathematical models of acute lymphoblastic leukaemia

Spontaneous mutation rate is sufficient by itself to generate the observed numbers of acute lymphoblastic leukaemia. If only one mutation were required then everybody would die of the disease. The observed rate places the numbers of mutations in the range two to three.

CELL CYCLE CONTROL⁽⁸⁾

Before considering specific types of mutation found in lymphoid neoplasia we need to understand the basics of cell cycle control (Figure 4).

(a) Phases of the cell cycle

After mitosis (M phase) the cell enters a period of rest (gap 1 or G1 phase) and cannot proceed further until the cycle control “gate” is opened by transcription factor. When permitted to do so, the cell enters the S or synthesis phase when DNA is replicated. This is followed by gap 2 (G2 phase) and mitosis again.

(b) Purpose of cycle control gate

Often termed the G1 checkpoint, this is the point at which mitotic restraint is applied. There are two essential purposes

Figure 4 Cell cycle and its control

of cell cycle control, the first one obvious, the second only recently appreciated:

- to control the rate of cell proliferation
- to stop mutated cells from cycling.

(c) Overview of control system

Proximal to the control gate the system consists of a sequence of stimulatory and inhibitory proteins, forming a “cascade” not unlike that of blood coagulation but allowing integration of signals rather than amplification. The system has two arms, a stimulatory mitotic drive pathway and a mutation control pathway which is inhibitory. If the inhibitory signals are preponderant then the control gate is not opened and the cell gets stuck in G1 (G1-arrest).

In reality the system is a good deal more complex than in Figure 4. There are, for instance, multiple types of cyclin and several more steps in the pathways. DNA repair encompasses several different systems requiring many genes. All these numerous control and repair genes are potential targets for random mutation.

(d) Mitotic drive

The driving force for mitosis is provided by the protein cyclin which fluctuates throughout the cycle and when high activates its partner enzyme cyclin-dependent kinase. The enzyme phosphorylates retinoblastoma protein. Retinoblastoma is a cycle suppressor which binds to the gate control key (E2F). When sufficiently phosphorylated, the retinoblastoma protein changes shape and releases the key.

(e) Mitotic inhibition by the mutation control pathway

Protein 53 (p53) is the central part of the mutation control

system⁽⁹⁾ and has been termed "the guardian of the genome". It is the most commonly mutated gene found in human cancers. P53 is able to detect DNA damage and responds by stimulating the production of p21 (a signalling protein) and inhibiting BCL-2 (apoptosis suppressor). P21 then inhibits cyclin-dependent kinase, which will cause the cell to undergo a G1 arrest; meanwhile p21 calls the DNA repair system into action.

(f) Consequences of G1 arrest of a mutant cell

A cell in which DNA damage has been detected will be held in G1 arrest, potentially for a prolonged period, until DNA repair is completed.

If DNA repair cannot be achieved, there is no way out of G1 arrest and the cell is destroyed by apoptosis.

(g) Apoptosis

Apoptosis is a programmed self-destruct mechanism which is built into every cell, but normally suppressed by the protein BCL-2⁽¹⁰⁾. When p53 inhibits BCL-2, the cell is free to commit suicide. It is somewhat sobering to realise that all our cells are primed and ready to self-destruct and only continue to live in the presence of a specific signal. The system has evolved to fail safe. BCL-2 gene is mutated in many forms of cancer, including follicular lymphoma

MUTATIONS FOUND IN LYMPHOID NEOPLASIA

(a) Introduction

Many different mutations have been recorded in the various types of lymphoid neoplasm⁽¹¹⁾, but several are found with a high degree of consistency⁽¹²⁾ (Figure 5). These are mostly translocations. Lymphocytes are especially vulnerable to translocation errors because of their unique method of generating antigenic specificity⁽¹⁾. Immunoglobulin genes are rearranged by a randomized process of DNA cutting, translocation and joining⁽¹³⁾.

Tumour Type	Mutation	Frequency	Result
Follicular lymphoma ⁽¹⁴⁾	Translocation 14;18	90%	BCL-2 activation
Mantle cell lymphoma ⁽¹⁵⁾	Translocation 11;14	70%	increased CYCLIN D1
Burkitt's lymphoma ⁽¹⁶⁾	Translocation 8;14	80%	C-MYC activation
Anaplastic large cell lymphoma ⁽¹⁷⁾	Translocation 2;5	60%	p80 activation (function unknown)
High-grade transformation of low-grade B cell lymphoma ⁽¹⁸⁾	p53	20% of high-grade B-cell lymphoma	inactivation of p53

Figure 5 Common mutations in lymphoid neoplasms

(bi) How does BCL-2 activation cause lymphoma?

BCL-2 activation will keep cells alive by inhibiting apoptosis. Mutant cells which refuse to die are a dangerous liability. In some situations normal cells must die; one such place is the germinal centre of the lymphoid follicles found in lymph nodes and spleen^(19,20).

(bii) What happens in germinal centres?

After creation in the bone-marrow virgin B-lymphocytes circulate for years in the body awaiting the first encounter with the antigen to which they have specific receptors. The meeting, should it occur, takes place in a lymph node where the captured antigen is displayed on specialised histiocytes. After recognition of its specific antigen the B-lymphocyte undergoes a burst of proliferation and creates a germinal centre from its progeny.

In the germinal centre BCL-2 expression is switched off because most of the proliferating B-lymphocytes must die. Only those with the most efficient antibody specificity are permitted to survive to become plasma cells and B memory lymphocytes⁽¹⁹⁾.

(biii) Consequence of a BCL-2 positive germinal centre

If a virgin B-lymphocyte has already undergone the 14;18 translocation causing permanent activation of BCL-2 it will probably have a long life, as usual, and no ill-effects will follow until the lymphocyte undergoes activation by specific antigen in a lymph node. The lymphocyte will then fill the germinal centre with mutant progeny and in the absence of apoptosis the process cannot be terminated. The progressive accumulation of germinal centres leads to follicular lymphoma although the process probably requires at least one further mutation.

(c) Effect of other lymphoid mutations

These can now be understood with reference to Figure 4. In mantle cell lymphoma the 11;14 translocation promotes the mitotic drive pathway by overproducing cyclin. C-myc in Burkitt's lymphoma stimulates the same pathway by an unknown mechanism. P53 inactivation will allow mutant cells into the cell cycle and effectively increase the mutation rate, by which cycle control is further degraded.

CONCLUDING COMMENTS

Parts I and II of this review have attempted to cover a substantial area of lymphocyte and cancer biology, and some concluding remarks are appropriate. Tumours of lymphocytes provide a diagnostic, therapeutic, and economic challenge. They form a stimulus for research and provide lessons for understanding cancer. Search for causes of lymphoid neoplasia is complicated by *embarras de richesses*. In my opinion the great majority of lymphoid neoplasms are caused by random mutations, the rate of accumulation of which increases exponentially with age due to degradation of mutation control systems. Viral infection may occasionally contribute one of the mutations required to produce acute lymphoblastic leukaemia. Hodgkin's disease and post-transplant lymphomas are probably caused by EB virus. Low doses of radiation have a powerful tumour-promoting effect *in utero*, but little thereafter.

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