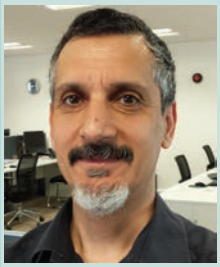
Li Fraumeni Syndrome: a new hypothesis



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Background

Li Fraumeni Syndrome (LFS) is a rare autosomal dominant hereditary cancer syndrome characterised by germline mutations of the TP53 tumour suppressor gene. The syndrome is associated with a range of cancers, particularly sarcomas, gliomas, adrenocortical and breast carcinomas, as well as other malignancies, particularly during childhood and early adulthood [1,2]. Among women with LFS, the most common is breast cancer, with a 49% risk of developing the disease by age 60 [3]. Overall the life-time risk of cancer is estimated at 52% by age 40 and 80% by age 50, and the life-time risk has been estimated at 100% in one study [2,4,5].

At least 70% of LFS patients have a germ line TP53 mutation, which remains the only confirmed genetic defect associated with the condition [2]. TP53 is primarily known for the central role it plays in the stress response of the cell to multiple insults, and is a key regulator of cell cycle arrest, apoptosis, senescence and DNA repair [6,7]. The pleiotropic roles of TP53 are still being elucidated and recent work points to p53 also having a role in ageing, immune response and cell metabolism [8-10].

There have been competing and evolving diagnostic criteria for TP53 gene testing in different countries and institutions. A simplified set of criteria has recently been published by Professor Nazneen Rahman of the Genetics and Epidemiology group at the Institute of Cancer Research. The decision-tree makes it clearer to clinicians presented with paediatric cancer patients, or adults with extensive personal or family histories of malignancy, whether a TP53 test is desirable [11].

Currently there are no cancer prevention strategies in place for LFS sufferers. Newly diagnosed patients are subject to varying levels of surveillance but are offered no practical steps to reduce the risk of developing malignancies other than bilateral risk-reducing mastectomy in women.

The main area of clinical research into LFS focuses on the development of more effective surveillance regimes for diagnosed sufferers. In particular, there is an increased emphasis on developing protocols for systematic scanning programs, including whole-body MRI scans. One example is the SIGNIFY trial currently underway in the UK, but similar trials are on-going in other parts of the world (http://clinicaltrials.gov/show/NCT01737255, http://clinicaltrials.gov/ct2/show/NCT01464086).

Puzzling Features of LFS

To date the common understanding has been that LFS patients are at greater risk of developing malignancies because of the accumulation of secondary mutations over and above the mutated TP53. However, there are a number of puzzling features of LFS that suggest that there are other important mechanisms involved in carcinogenesis in addition to damage to DNA repair mechanisms or missing tumour suppressor activity.

The first and most obvious puzzle in LFS is that the syndrome is characterised by unusual patterns of cancer incidence. For example, the preponderance of soft-tissue and bone sarcomas, adrenocortical carcinomas, gliomas and other rare cancers. But there is no evidence of increased risk of tobacco-associated lung cancer or other malignancies associated with environmental toxins or occupational hazard, as would be expected if damaged DNA repair mechanisms was the primary outcome from loss of TP53 function [12].

Clinical evidence exists that LFS patients have shorter telomeres than age-matched non-LFS individuals and that children with LFS have mean telomere length shorter than unaffected parents or siblings [13,14]. Furthermore, shorter telomere length is associated with a younger age of cancer onset in LFS patients, and there is convincing evidence of increased telomere attrition in succeeding generations [15]. In many respects, telomere length can be seen as an indicator of biological aging that is independent of chronological age, and that shorter telomere length corresponds to greater biological age [16].

There are other aspects of the non-cancerous LFS host that correspond, in some respects, with characteristics of a more aged phenotype, including significantly increased indicators of oxidative stress and a remarkable degree of down-regulation of caveolin-1 (cav-1) [17,18]. The latter finding, comparing LFS carriers to non-carriers within affected families, is especially noteworthy in that loss of cav-1 is generally accepted as a marker of premature aging in cav-1 knock-out mice.

Analysis of non-malignant fibroblasts and other cells derived from LFS display unusual patterns of senescence and some of them can undergo spontaneous immortalisation in vitro. Where control fibroblasts from skin biopsies underwent senescence in the normal way, some of the fibroblasts from a number of LFS patients entered a long period of slow growth and replicative senescence during which they showed altered morphology, chromosomal damage, including aneuploidy and telomeric association (contact between two chromosomes at their terminal ends), followed by escape from senescence, and the resumption of cell division and replication. Spontaneous immortalisation of human fibroblasts almost never occurs in cultures from non-LFS patients [19,20].

The "Two Compartment LFS" Hypothesis

A new hypothesis is therefore proposed that links these diverse clinical and epidemiological phenomena of the host environment in non-cancerous LFS patients, suggesting that the host is 'primed' with the pre-conditions for cancer to develop according to the 'two compartment tumour metabolism' theory developed by Professor Michael Lisanti and his colleagues [21].

The 'two compartment tumour metabolism' theory is a new paradigm that describes a metabolic shuttle between autophagic cells in the tumour stroma and tumour cells [22,23]. The theory proposes that cancer cells induce oxidative stress in the stroma by secreting hydrogen peroxide into the surrounding tissues. Cancer-associated fibroblasts (CAF) respond to this environmental challenge by activation and entry into an autophagic state, undergo mitophagy,

Table 1. Characteristics of accelerated host aging in tumours and in the non-cancerous LFS host

Characteristic	Two Compartment Tumour Model	Non-Cancerous LFS Host
Senescent Fibroblasts	✔	✔
High Oxidative Stress	✔	✔
Loss of Stromal Cav-1	✔	✔
Tumour Cells Resistant to Autophagy	✔	✔

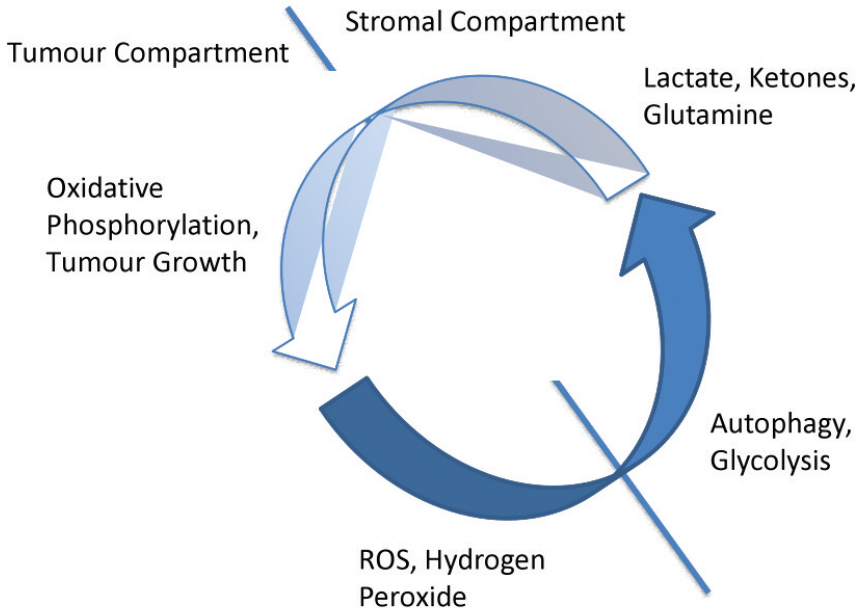

Figure 1. The metabolic shuttle between autophagic stromal cells (glycolytic metabolism induced by ROS), delivering high energy fuels to coupled tumour cells (depending on oxidative phosphorylation).

developing mitochondrial dysfunction and experiencing a shift of metabolism towards aerobic glycolysis. This metabolic shift results in the production of high energy by-products, l-lactate, ketones, glutamine and other mitochondrial substrates, that the tumour cells require to drive growth [24,25].

At the heart of this relationship between tumour cells and the surrounding stromal tissues is the autophagic response to oxidative stress [26]. There is a relationship between cellular senescence and autophagy, suggesting that they are part of the same "autophagy-senescence transition (AST)", and that they both promote the anabolic growth of cancer cells. It also links aging and cancer in a radically new way, suggesting that cancer is a disease of 'accelerated host aging' in the tumour stroma [22,27].

An important hallmark of the 'two compartment' theory is a loss of stromal cav-1 and a corresponding upregulation of cav-1 in tumour cells (i.e. the effects of cav-1 are compartment-specific) [28]. Loss of stromal cav-1 expression is a key indicator of the effect of immortalised epithelial cells on adjacent fibroblasts [29,30]. The characteristics of the accelerated aging model are summarised in Table 1, which also lists the corresponding features of the non-cancerous LFS host. This new LFS hypothesis suggests, therefore, that LFS affected individuals are 'primed' with the pre-conditions for 'two compartment tumour metabolism' to take place. Once cancer is initiated, the host environment is already in a state where stromal fibroblasts respond to tumour cells by becoming activated and moving into a state of autophagy, mitophagy and switching metabolism to aerobic glycolysis, thereby feeding the tumour cells with the high energy by-products of this form of metabolism. In short, cancer in LFS patients rapidly moves to a state of 'two compartment' tumour metabolism.

Clinical Implications

This shift in emphasis from a focus on accumulated DNA damage to cell-cell interactions and accelerated aging has important therapeutic implications for LFS, of which the most important single consequence is the idea that altering certain aspects of the LFS host environment can markedly reduce the risk of cancer initiation and progression. Chief among these is the disruption of the preconditions for 'two compartment tumour metabolism' to take place.

The new hypothesis outlines 3 targets for this approach to active cancer reduction strategies for LFS patients:

1. Inhibition of senescence in stromal cells
2. Induction of autophagy in tumour cells/inhibition of autophagy in stromal cells
3. Disruption of the metabolic shuttle between stroma and tumour

Preclinical evidence exists in support of each of these strategies. For example, Komarova and colleagues [31] showed that the mTOR inhibitor of rapamycin, which inhibits cellular senescence, increased lifespan and decreased the incidence of spontaneous tumours in p53 + /- mice. The effect was stronger when started early in life, suggestive of a systemic effect in the host rather than in direct anti-tumour activity.

Autophagy has compartment-specific effects and the metabolic shuttle (Figure 1) can be interrupted by inducing autophagy in tumour cells, which are then unable to metabolise the high energy fuels from autophagic stromal cells. Alternatively, stromal cells can be stopped from entering autophagy (and hence from generating the high energy fuels required by the coupled tumour cells) by the use of the autophagy inhibitor, chloroquine.

Another prediction of the hypothesis is that cancer incidence in LFS can be reduced by restriction of glucose supply, which primarily feeds cells in the stroma. Options for altering the availability of glucose include dietetic alterations or pharmacological interventions. Supporting evidence for a metabolic influence in LFS carcinogenesis is provided by work on p53 + /- mice, which showed that calorie restriction in adult animals delayed the development of cancer [32].

Chief among the pharmacological interventions is the use of the anti-diabetic drug, metformin, which targets many of the pathways affected by dietary caloric restriction, including AMPK, mTOR and IGFR [33]. In the context of the LFS phenotype, it would have dual effects. First it acts to restrict the supply of glucose to activated stromal cells through the inhibition of hepatic glucose production. Second it can act to block mitochondrial oxidative phosphorylation in tumour cells, thus starving cancer cells through two distinct pathways.

Clinical Implications

This hypothesis opens the door to active chemo-preventive strategies in terms of autophagy inhibition, steps to reduce oxidative stress, and so on. Drugs such as metformin, chloroquine and other agents with low toxicity, including anti-oxidants, may also be worthy of further investigation in LFS families. ■

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