Effects of food on metabolic regulation and disorders

Authors
Stephanie Blum*, Bruce German*†, Peter van Bladeren* & Johannes le Coutre*

*Nestlé Research Center, CH 1000 Lausanne 26, Switzerland

†Department of Food Science & Technology, University of California, Davis, California 95616, USA

The diseases that make up the cancer family involve multiple biological processes. As research gradually unravels this complexity, many aspects of the human condition must be understood to reduce individual risk, to intervene in the initiation and development of cancerous processes, and to reverse them. Vast resources are necessary for success, and the implications of scientific research must extend to aspects of human lifestyles, including diet. Scientists, clinicians and public-health officials agree that successful approaches to solving the mysteries of cancer will take diet into account. As the leading industrial food-research institution, Nestlé recognizes its important role in this effort, and invests resources in many aspects of nutrition and health, in order to contribute to scientific knowledge and to translate it into practical nutrition solutions as rapidly as possible.

Nestlé has built competencies in fundamental research that contribute to the global battle against cancer in the areas that are most influenced by diet. Nestlé researchers are asking questions about the role of food in protecting cellular biomolecules from damage, in the cellular processes of detecting and responding to external stimuli, in immunological processes, in reducing chronic inflammation and subsequent damage, and in promoting molecular, cellular and tissue repair. Furthermore, diet is important to the overall health of patients. Both the processes of cancer and the related therapeutic strategies affect the nutritional and metabolic status of individuals who are fighting the disease. Basic physiological processes that sense food and regulate intake are also disrupted during cancer. Clinical nutrition products are needed to assist patients in maintaining their critical tissues and functions, to provide support for their unusual nutrient demands, and to help regulate their metabolism and immune systems. Nestlé actively pursues research and innovation strategies to provide appropriate nutrition to those who are affected by the disease and its treatment.

The Nestlé Research Centre in Lausanne, Switzerland, is committed to addressing these critical issues by conducting internal research, coordinating worldwide collaborations and hosting international symposia. The recent Nestlé international nutrition symposium on nutrition and cancer, and the support of the current insight are examples of our ongoing commitment.

Nestlé's focus on the mechanisms of carcinogenesis

[AU: is the edit OK?]
[AU: please add a sentence explaining to the reader Nestlé's interest in this topic] The complexity underlying cancer is becoming more obvious as scientific research continues to unravel the molecular processes of carcinogenesis and malignant transformation, and the link with other diseases. Science aims to identify the precise molecules and mechanisms that lead to cancer, which could be suitable targets for early diagnosis, therapeutic intervention and prevention, all of which relate in some way to diet and therefore guide the Nestlé research program (Fig. 1).

Loss of cell-cycle control, cyclins and cyclin-dependent kinases (CDKs). The hallmark of every cancer is the combination of unregulated cell growth and proliferation, as well as invasion and eventually malignant transformation, of a few cells in a given tissue. As described by Hanahan and Weinberg[9], [AU: please cite references [1–8] before reference [9]] six traits lead to carcinogenesis: self sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless proliferation potential, sustained angiogenesis and evading apoptosis. Establishing these traits focused the attention of Nestlé on the inevitability of multiple intervention strategies, and on the potential for diet to have effects at many discrete sites and, therefore, to form part of the solutions.

Central to all cancerous processes is the

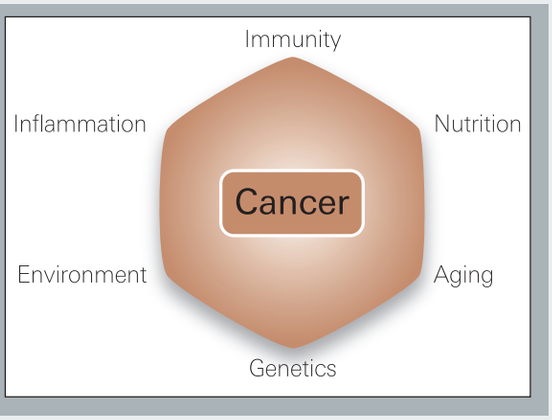

Figure 1 | Intrinsic and extrinsic factors determine the complexity of cancer in a network of interdependencies. The significance of these factors over a lifetime is greater for the evolution of cancer throughout its proliferation than for therapy. [AU: please clarify the meaning here] Being the sole parameter of direct patient choice, nutrition might also have secondary effects on cancer proliferation via immunity, inflammation and healthy aging.

cell cycle. Sustained integrity of the cell cycle is mandatory for maintaining control of growth and proliferation. The essential regulators across all cellular species are cyclin A, cyclin B, cyclin D and cyclin E, which form functional heterodimers with CDKs that subsequently promote consecutive cell-cycle steps. Several checkpoints related to cyclin–CDK complexes have been identified, ranging from the controlled expression of specific transcription factors and further cyclins, to enzymes that are necessary for DNA replication[13]. [AU: please cite references [10–12] before reference [13]] Moreover, active cyclin–CDK complexes phosphorylate other proteins to coordinate and synchronize all cellular processes prior to cell division.

Targeted protein degradation. The scientific world is accustomed to surprises. Even so, it came as a shock when, after decades of research focused on the molecular mechanisms controlling protein synthesis, targeted and active degradation of proteins was discovered to be an equally important feature in controlling the cell cycle and proliferation. The frailty of cellular homeostasis is exemplified by the action of ubiquitin-mediated proteolysis. Ubiquitination is a remarkably complex and widespread process, which is responsible for tagging proteins to be degraded in the proteasome. Clues to its importance for health came from the discovery of rare pathologies, such as Angelman syndrome and von Hippel–Lindau syndrome, caused by malfunctions of the ubiquitin pathway[14]. It was only a matter of time before the process of ubiquitination was recognized as a key aspect of cancer development in which cell-cycle control can be lost by the failure to degrade an oncoprotein or by accelerated degradation of a tumour-suppressor protein (Fig. 2).

Ubiquitin is directly linked to the sensitive equilibria of regulatory proteins driving the cell cycle; in particular, on activation of cyclin–CDK complexes, molecules that function as S-phase inhibitors are targeted for degradation. The importance of ubiquitination and its regulation throughout biology became clear to Nestlé researchers when it was discovered that this regulatory network was conspicuously downregulated in the mammary epithelium during lactation and participated in the successful synthesis of milk proteins[11]. This led Nestlé to ask whether the growing understanding of the regulation of ubiquitination during lactation could be translated into targets for dietary intervention.

The ubiquitin system of targeted protein elimination is therefore involved in cell-cycle checkpoints and in apoptotic mechanisms, alterations of which influence carcinogenesis and cancer progression. As discussed below, the intricately controlled cell-cycle checkpoints simultaneously represent entry points into carcinogenesis when proto-oncogenes turn into oncogenes.

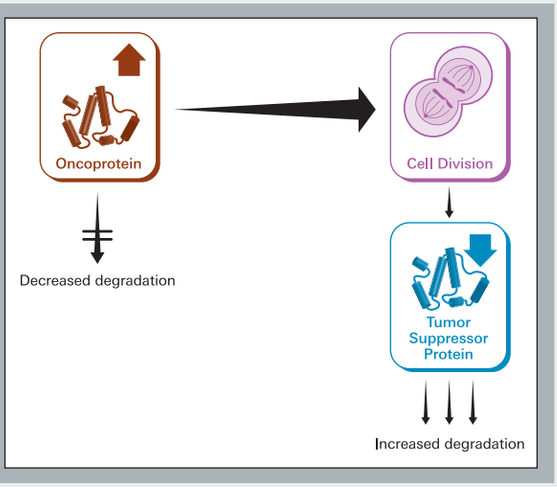

Figure 2 | Targeted and active protein degradation via ubiquitin tagging and hydrolysis in the proteasome maintains a delicate equilibrium in a population of proteins, such as potentially carcinogenic oncoproteins and antiproliferative tumour-suppressor proteins. Figure reproduced courtesy of Avram Hershko. [AU: do we have permission to use this figure?].

Viral transformation, oncogenes and carcinogenesis. In 1971, Robert Huebner and George Todaro postulated an intriguing mechanism describing how retroviral oncogenes directly facilitate human cancer, in what became known as the viral oncogene hypothesis[10]. At the centre of this theory was the idea that the cells of many, and perhaps all, vertebrates contain information for producing RNA viruses. The theory postulates that "the viral information (the virogene), including that portion responsible for transforming a normal cell into a tumour cell (the oncogene), is most commonly transmitted from animal to progeny animal and from cell to progeny cell in a covert form. Carcinogens, irradiation and the normal aging process all favour the partial or complete activation of these genes".

Yet, in 1976, J. Michael Bishop, Harold Varmus and Dominique Stehelin disproved the Huebner–Todaro hypothesis, showing that human cells had their own oncogenes, many of which would eventually be implicated in cancer[7].

Following this stimulating beginning, scientists at Nestlé have recognized that the knowledge gained from their long experience of studying the interactions between diet, nutrient deficiencies and the emergence of infective viruses also has great relevance to the study of the development of cancer. Scientists around the world have continued to pursue these ideas and we now know that many normal genes can function as proto-oncogenes, which, on mutation or overexpression, become carcinogenic oncogenes. The gene products encoded by an oncogene are themselves often involved in the cell cycle, cell growth and differentiation. One captivating example of the fine-tuned supervision of cell-cycle checkpoints was given by Charles Sherr at the fourth Nestlé international nutrition symposium, as he elegantly unravelled the roles of the CDK inhibitor 2A (CDKN2A)–CDKN2B gene cluster in tumour suppression and cell-cycle control. The role of these tumour-suppressor networks in a wide range of biological processes in which diet is involved cannot be underestimated, as recent population-wide genotype/phenotype association studies make a clear link between the CDKN2A–CDKN2B cluster and type 2 diabetes[15].

The importance of viruses in the development of cancer has come full circle now that viral infection is recognized to contribute to ~15% of all cancers worldwide[20]. [AU: please cite references [16–19] before reference [20]] The human papilloma virus was unequivocally established as the single cause of preventable cervical cancer carcinogenesis. This remarkable scientific achievement has been translated into public-health prevention measures through vaccinations, which are increasingly becoming available around the world. The first vaccination against cancer, however, was indirectly implemented by preventing hepatitis B and hepatitis C, which are clearly implicated in the development of liver cancer and involve food-borne vectors. Increasingly, infective causes of cancer are being identified. For example, the Epstein–Barr virus has a causative role in Burkitt lymphoma, nasopharyngeal cancers and lymphomas, similar to the role of the human T-cell lymphotropic virus in adult T-cell leukaemia. Likewise, chronic *Helicobacter pylori* infection is the leading cause of gastric cancer worldwide.

Nestlé's interest in chronic inflammation

[AU: please add a sentence explaining to the reader Nestlé's interest in this topic] A growing body of experimental data is revealing the relationship between pathological processes of infection, inflammation and cancer. Persistent infections within the host induce chronic inflammation. Leukocytes and myeloid-cell infiltrates produce reactive oxygen species and reactive nitrogen species, which are normally used to fight infections. These species form reactive metabolites that can act as mutagenic agents, therefore inducing DNA alterations and genomic instabilities. Occurrences of mutations in p53 are similar to those

of tumours in chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease. The organs that are most susceptible to chronic inflammation with consequent tumour development are the lungs, bladder, oesophagus, pancreas and gastrointestinal tract. Indeed, the strongest association of chronic inflammation with malignant disease is seen in colon carcinogenesis arising in individuals with Crohn disease and ulcerative colitis. Nestlé has been studying diet and intestinal inflammation for decades to understand how to design foods that can prevent and ameliorate these conditions[4].

Further clinical evidence of the importance of inflammation during neoplastic progression comes from the 40% reduction in colon cancer risk observed in long-term users of aspirin and non-steroidal anti-inflammatory drugs, and their preventive potential for lung, oesophagus and stomach cancer[2].

In addition to epidemiological data across populations, genetic polymorphisms in the tumour necrosis factor (TNF), interleukin-1 (IL-1) or Toll-like receptor (TLR) genes, which result in increased levels of pro-inflammatory cytokines, have been associated with poor diagnosis and disease severity in non-Hodgkin lymphoma, and in gastric and prostate cancer[17,18].

A central role in linking inflammation and immunity to cancer development and progression has been attributed to nuclear factor-κB (NF-κB). This belongs to a family of evolutionarily conserved eukaryotic transcription factors with pivotal roles in regulating innate and adaptive immune responses. Target genes of the NF-κB pathway, such as TNF-α, IL-1β, IL-6 and IL-8, were shown to be associated with tumour development and progression in humans and mice. The inhibitor of NF-κB kinase-β (IKK-β)-dependent NF-κB pathway strongly relates to pro-oncogenic functions. [AU: is the added full name correct?] It is triggered by bacteria, viruses and pro-inflammatory cytokines, resulting in the activation of the IKK complex (the IKK-α/IKK-β/IKK-γ heterotrimer), which in turn phosphorylates the NF-κB-bound inhibitor of κB (IκB). Phospho-IκB (p-IκB) [AU: are the added full names correct?] undergoes ubiquitination by proteasomal degradation, and the NF-κB heterodimer (RelA/p50) translocates into the nucleus, initiating transcription of a range of target genes. Besides microenvironmental alterations due to growth factors, pro-inflammatory cytokines and chemokines, NF-κB activation also results in the expression of inducible nitric oxide synthase (iNOS or NOS2), cyclooxygenase-2 (Cox-2) and anti-apoptotic genes, such as B-cell lymphoma-XL (BCL-XL), growth arrest and DNA damage-inducible protein-45β (GADD45β) and superoxide dismutase-2 (SOD2), thereby promoting the survival of pro-malignant cells under hypoxic conditions. [AU: are the added full names correct?] The central role of the NF-κB pathway and its demonstrated sensitivity to exogenous factors makes it a strategic target for research into dietary influences on inflammation.

Understanding these microenvironmental alterations in molecular detail has led to the realization that the severity of inflammation and carcinogenic progression can be further enhanced by the cross-talk of transcription factors. It was demonstrated that the synergistic action of NF-κB with the hypoxia-inducible factor-1α (HIF-1α) remodels the local microenvironment in the epithelium, with a subsequent phenotypically silent accumulation of stromal inflammatory cells[16].

More examples of specific signalling networks in disease are emerging. For example, a new study reveals the mutual repression between the xenobiotic nuclear receptors (PXR/SXR) and NF-κB, and provides a molecular link between drug metabolism, inflammation and immune suppression[33]. [AU: please correct the reference number]

In the absence of chronic inflammation, inflammatory processes in the surrounding tissues, such as stromal cells or tumour-infiltrating macrophages (TAMs), neutrophils and T cells, were shown to be important determinants of tumour development[12]. These inflammatory cells provide growth factors, including TNF-α, IL-6 and vascular endothelial growth factors (VEGFs), thereby promoting tumour growth and vascularization.

Based on experimental evidence, activation of the classical (but also alternative) NF-κB pathway seems to be relevant at all stages of cancer progression, thereby providing a valid target for novel intervention strategies. Current prospective trials favour the combination of NF-κB inhibitors with other targets, such as apoptosis-inducing drugs, as experimental studies have shown that inhibition of the NF-κB pathway alone is not sufficient for tumour regression.

A better understanding of the tumour-promoting potential of inflammation and the role of innate immunity might lead to the development of new and improved preventive/therapeutic combinatorial approaches, including the long-term use of dietary che-

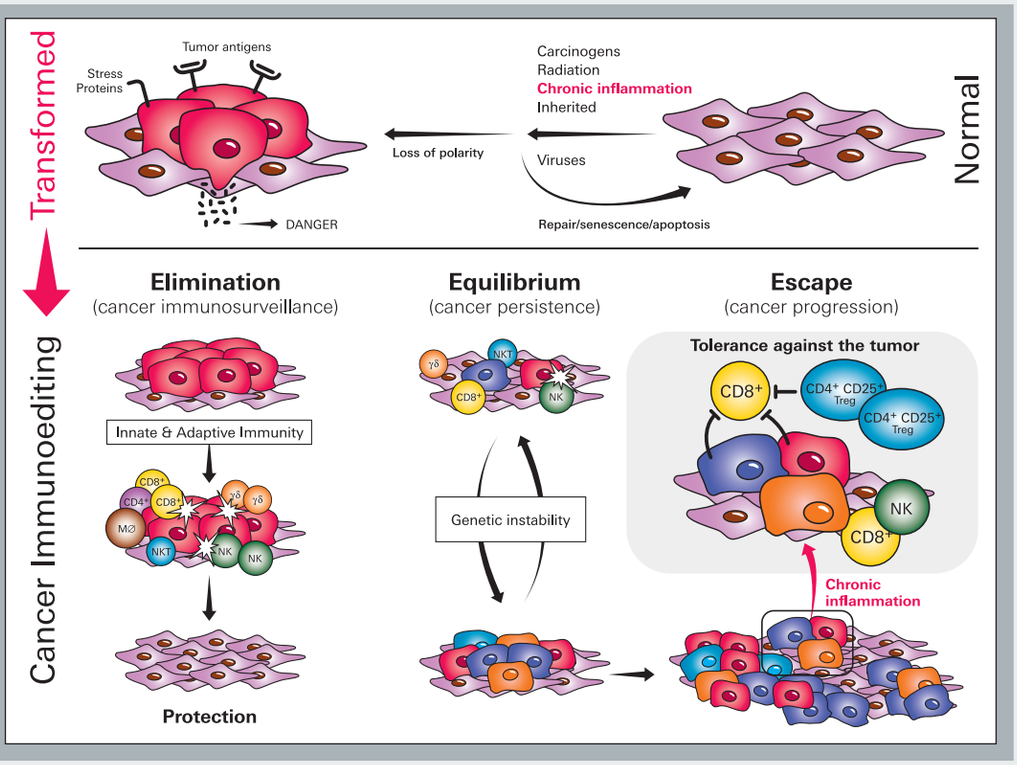

Figure 3 | **Hypothesis of cancer immunosurveillance/immunoediting. Via the transition of normal cells to pre-malignant and tumour cells, stress proteins or tumour antigens are expressed; these can then be recognized by cells of the innate and adaptive immune system, thereby leading to destruction and elimination of the tumour cells (immunosurveillance). In the equilibrium state, the tumour persists, but is prevented from expansion by immune pressure. In the escape phase, the tumour succeeds in growing due to immune exhaustion or as a result of emerging tumour variants that allow it to evade immune suppression. Figure adapted from Ref. 5. [AU: do we have permission to use this figure?].**

mopreventive compounds as modifiers of the NF-κB pathway[3].

Nestlé's work on cancer immunosurveillance

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Research at Nestlé into the extensive interaction between diet and immunity considers that the former could influence carcinogenic processes indirectly by its actions on immune functions. In particular, Nestlé research has previously established that diet affects the development, integrity, activity and maintenance of immune surveillance through essential nutrients, bioactive chemicals, the composition of the intestinal microflora and the fermentable oligosaccharides that feed these bacteria. The capacity of the immune system for recognition is not limited to 'self' versus 'non-self' in terms of failures to recognize pathogens or to tolerate allergens, but encompasses the differences between 'self' and 'transformed self'. This same principle led other scientists to the hypothesis of cancer immunosurveillance stating that the immune system can protect the host against cancer cells. Over the years, this hypothesis was supported by different animal models that were defective in innate or adaptive immune functions, confirming that immunodeficient mice developed more spontaneous and carcinogen-induced tumours[5]. The subsequent formulation of the cancer immunosurveillance/immunoediting hypothesis outlined a role for the immune system in eliminating immunogenic tumour cells actively, although it also stressed the role of immunity in promoting the outgrowth of less-immunogenic tumour cell variants (immunoediting) followed by the evasion of tumour suppression (escape). The concept evolved from numerous observations that the tumours from immunocompetent and immunodeficient hosts exhibit different immunogenic properties. Paradoxically, tumours that developed in the presence of an intact immune system (as usually occurs in humans) formed progressively growing tumours when transplanted into immunocompetent recipients. By contrast, tumours originating from immunodeficient donors were rejected when transplanted into immunocompetent recipients (but not immunodeficient animals)[5]. The immunogenicity of a tumour therefore reflects its original immunological environment, which is responsible for imprinting the emerging tumour repertoire (Fig. 3).

Clinical data have led to a growing appreciation that cancer immunosurveillance and immunoediting also occur in humans. The incidence of cancers with non-viral origins is rising in immunosuppressed transplant patients, and there is a growing correlation between intra-tumoral $CD8^+$ cytotoxic and tumour-protective regulatory T cells ($CD25^+FoxP3^+$), with the latter causing T-cell tolerance leading to immune escape of the tumour[5]. In a recent large cohort study, the type, density and location of immune cells in human colorectal cancer were shown to be predictive of clinical outcome, further emphasizing the role of the adaptive immune response in controlling tumour growth and recurrence in established disease[8]. Nestlé's understanding of the effects of diet on immune surveillance could be translated into improvements in cancer risks, offering attractive opportunities for the future of food development.

The reported functional players in immunoediting comprise innate immune cells, such as γδT cells, natural killer (NK) T cells (which use expression of the NKG2D receptor to recognize stressed cells [AU: is the edit OK?]) and αβ T cells. Immune mediators, such as type I interferons (IFN-α/β), IFN , perforin, TNF-related apoptosis-inducing ligand (TRAIL), signal transducer and activator of transcription-1 (STAT1), IL-12 and IL-23, are key players in the decision process between immunosurveillance (tumour regression) and immunoediting (tumour progression). However, whether immunosurveillance/immunoediting and tumour-promoting inflammation are two mutually exclusive processes or potentially overlapping immune algorithms has not yet been established.

Future research is needed to characterize the cells and factors that orchestrate this specific immune response. Understanding the molecular basis of the decision-making process and the potential influence of exogenous immune-response modifiers, including the role of dietary components, is a clear priority for research and its translation.

The complex pathways of inflammation and immune activation are linked through interaction/signalling networks the functions of which are responsible for the high plasticity of immune cells in rapidly adapting to changing microenvironments. It will be important to

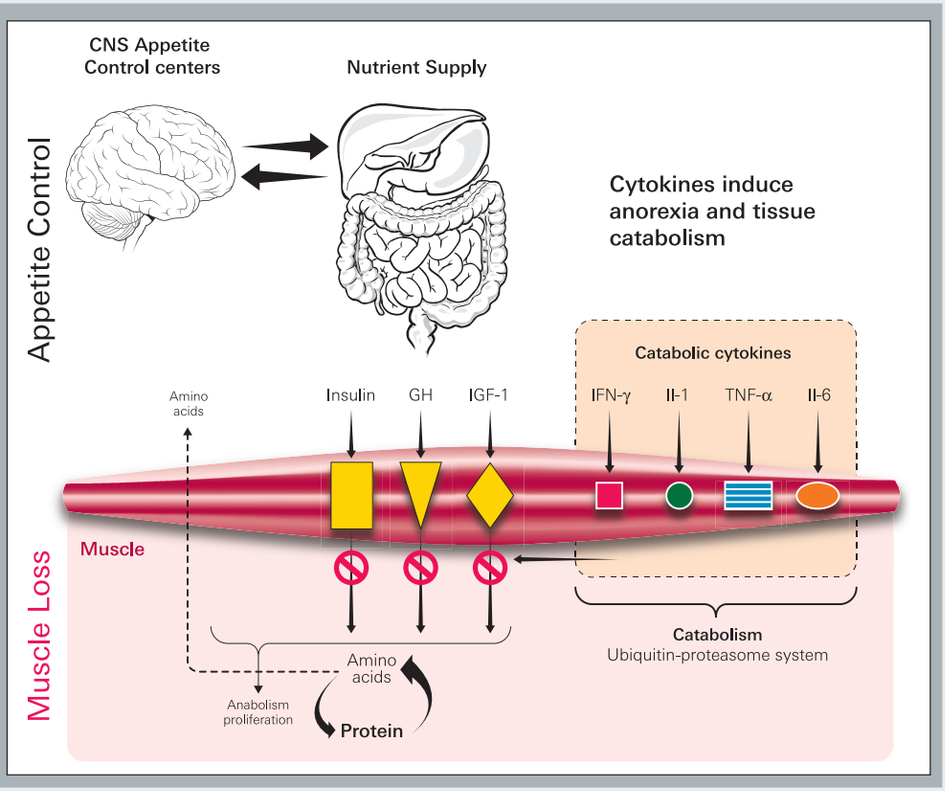

Figure 4 | Pathophysiology of cancer-associated cachexia. This is characterized by two principle processes. The first is the loss of lean body mass (especially muscle) following a hypermetabolism/hypercatabolism syndrome driven by alterations in circulating cytokines (TNFα, IL-1, IL-6 and IFNγ) that induce tissue catabolism. The second is the failure of food intake, resulting in the depletion of physiological reserves of energy and protein.

resolve the linear and temporal sequences of such signalling webs. Computational analysis of data sets derived from systems biology will be a means to model such complex networks and their principle 'modules'. This will help to identify major decision points in interconnected pathways and commonalities of dysregulated processes in pathophysiological conditions. Moreover, such analysis will provide an educational base for new target identification, and potential points of pharmacological and nutritional intervention.

Nestlé's focus on the nutritional needs of cancer patients

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[AU: please add a sentence explaining to the reader Nestlé's interest in this topic] The processes of cancer are not simply passively debilitating to those involved, but elicit devastatingly active tissue destruction (cachexia), particularly of the lean body mass. Tissue loss is due to both a systemic hypermetabolism/hypercatabolism syndrome driven by imbalanced cellular signalling systems and, typically, a simple failure of food intake[1]. Furthermore, many therapeutic approaches compromise the desire, or ability, of patients to successfully consume, digest and absorb sufficient food to meet their elevated nutritional requirements. These problems mean that the cancer patient requires nutritional support with a unique perspective that considers all other background health issues. [AU: is the edit OK?] For example, the traditional view of the cachectic state is one of overwhelming caloric insufficiency. Yet, many cancer patients who are beginning therapy today are physically overweight, and remain so despite losing substantial proportions of their lean muscle mass to the point of sarcopaenia. Nestlé research has revealed that foods for cancer patients must be formulated to guide metabolism, reinforce immunity and enhance tissue repair, while also being easily digested and absorbed, and remaining appetizing. The Nestlé approach brings together the following: the sciences of nutrition for metabolism support and immunity; the sciences of food to structure food components for rapid and complete digestion; the sciences of intestinal physiology to ensure absorption; and the sciences of neurophysiology and sensation to ensure positive sensory stimulation (Fig. 4).

Nestlé's vision for the future

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Cancer research is revealing that the members of this family of related diseases represent an insidious dysregulation of the processes of life itself. The complex scientific breakthroughs that are emerging worldwide promise at last to address these diseases, and will equally challenge our ability to continue translating scientific discoveries into action. Diet has an influence on every step of the processes of cancer, from protecting regulatory biomolecules during its initiation, to supporting the immune system while detecting it and rebuilding the muscle tissue lost while fighting it. [AU: is the meaning OK here?] Nonetheless, the ability of foods to act alone, or even to complement other approaches at any of these points, remains to be determined. Nestlé is committed to pursuing the scientific knowledge necessary to understand how to leverage nutrition, and how to build effective solutions for therapy and prevention.

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