Original Article

Biomarkers, yesterday, today and tomorrow: the basis for health claims

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The development of useful and accurate biomarkers for predicting outcomes of food based interventions is becoming more and more important, given the emphasis being placed on ingredients in foods contributing to disease risk reduction and optimal health promotion. With the human genome now laid bare, opportunities abound to barcode individuals with their risk profiles. The massive increase in DNA sequence information together with the development of new technologies such as genomics, proteomics and bioinformatics, has resulted in a much greater capacity to determine individual risk profiles. Screening for biomarkers at the gene or protein expression level using microarray technology has the potential to identify new biomarkers for disease diagnosis. Whether these techniques will enable a better understanding of food–gene interactions to permit health claims rather than better therapeutic treatment (at high economic cost) remains to be demonstrated.

Key words: foods, genomics, health claims, microarray technology, nutrients, proteomics.

Introduction

Biological markers or biomarkers reflect a step in the process between exposure and disease that can be quantified. As far as it is feasible, biomarkers should be on the causal pathway between the exposure and the outcome (Fig. 1).

Biomarkers reflect biological intermediates that relate to exposure, to disease itself, or to susceptibility to disease. The promise of biomarkers is that they may provide a more accurate measure of these entities than 'old-fashioned' methods. For example, rather than judging occupational exposure to a food or ingredient via a questionnaire, one might be able to measure levels of protein or DNA adducts. Instead of waiting for many years for clinically obvious disease to develop, one may be able to quantify abnormal cells or nuclei or another surrogate biomarker as a precursor of disease, and intervene before the disease fully develops. Surrogate disease endpoints are also useful in reducing the time taken for evidence to accumulate to support a health claim.

Biomarkers are measured using laboratory technology, and whenever any entity is measured, the possibility of error is also present. Therefore, before any biomarker is adopted and used, it needs to be validated in a broad sense; this includes the measurement of validity and reliability.[1] The former is closely related to the degree of bias in the measurments, while the latter deals more with the precision of measurements. The validation of biomarkers passes through two stages, development of the assay itself, and its technical properties followed by characterization of the biomarker in the population. The latter characterizes the properties of the biomarker in the population of interest and usually address the prevalence in the population including level of risk, exposure and prevalence in normal populations; dose–response studies; persistence of the marker and its specificity.

Biomarkers and health claims

With respect to Health claims for foods the specific needs for biomarkers have been well summarized in a document produced by Health Canada.[2] Three types of requirements for biomarkers are suggested as essential for the different types of potential claims, surrogate disease end point markers to assist with risk reduction claims, biomarkers that reflect maintenance of optimal health for nutrient function claims, and biomarkers that reflect dietary intake for validation of effectiveness. Biological markers of dietary intake would remove much of the difficulties encountered with all of the current dietary intake survey methodologies. These difficulties include poor correlation between methods, difficulty in knowing the absolute amounts eaten of any particular food/nutrient, poor specificity and precision of measurement. While epidemiological studies of the cohort and case-control type can overcome some of these problems, providing the error is randomly distributed between study populations, an accurate estimate of amounts eaten would be needed to deliver on the promise of disease risk reduction claim about a class of food or nutrient enriched food. Ideally, appropriate biomarkers of food intake should discriminate between past

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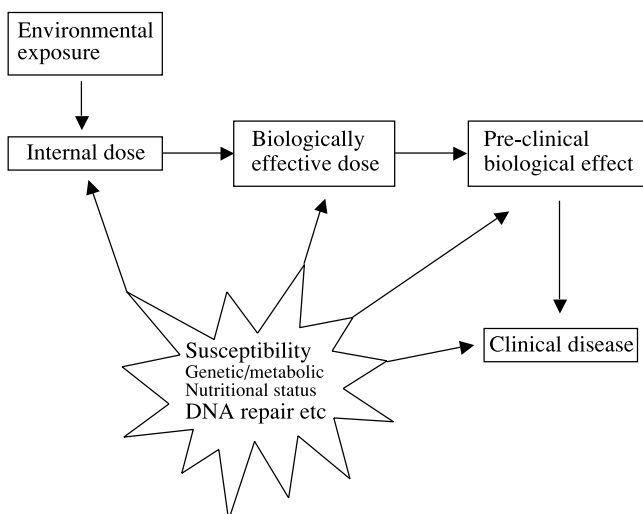Tel:+61 2 49215648; Fax: +61 249216984
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Figure 1. Factors that influence individual susceptibility to disease.

(long-term) and present (short-term) intakes, measure intake with precision and reflect changes in the consumption of the food substance in question.[2] Assuming such a marker existed, the extent to which it would reflect the amounts present in the diet would depend on factors such as those that affect absorption, metabolism, distribution, and excretion.

As indicated above biological markers need to meet a number of requirements before their use can become widespread. They should be measurable in easily accessible material, be linked to the outcome involved in the biological process being studied, represent relatively immediate outcomes, which can be used to assess interventions in a reasonable time and be rigorously validated.[2]

Validated existing markers
One of the oldest biomarkers is that of plasma low-density lipoprotein (LDL) cholesterol which acts not only as a biomarker of saturated fat intake but is also part of the disease process en route to the endpoint of coronary heart disease. Because LDL cholesterol levels are related to coronary events, it is a true biomarker on the causative pathway. However, its specificity is not perfect, predicting less than half of cases due to the multifactorial nature of cardiovascular disease (CVD). Homocysteine levels may be another biomarker of use in CVD.[3] Here the link is with micronutrient intake of folate, B_6 and B_{12}. In considering biomarkers, the construct into which they fit is an important consideration. A construct is a set of ideas that represent knowledge of how a complex event occurs. It is theoretical but shaped by observation and often results from an inability to directly measure the event of interest. For example, there is a hypothesis that oxidative stress is a key mediator in coronary heart disease,[4,5] even though direct measurement of oxidative events in the target tissues is not possible. For homocysteine to be a protective biomarker such a construct would require; subjects with a low exposure to folate/B6/B12 should have higher levels of homocysteine than those with high exposure to these micronutrients, and there should be a rise in the biomarker when exposure is reduced.

Biomarkers in cancer prevention are many and varied and may or may not have good construct validity. For colorectal cancer and nutrition, there are three available in precancer events, epithelial proliferation, aberrant crypt foci and adenomatous polyps.[6] The latter has been criticised for lack of sensitivity, in that a proportion of adenomatous polyps do not go on to become cancerous.[7] A specific marker that identified the proportion that do go on to develop cancer would be welcome. Genotypic biomarkers may be the answer as they could potentially identify that subgroup.[8] With the human genome now laid bare, opportunities abound to barcode individuals with their risk profiles.

Future markers
The massive increase in DNA sequence information together with the development of new technologies such as genomics, proteomics and bioinformatics, has resulted in a much greater capacity to determine individual risk profiles. Knowledge of gene and protein expression regulation following exposure to biologically active compounds make it possible to identify changes in biochemical pathways. Assuming gene and protein expression changes precede change at the tissue level, protein profiling under conditions of exposure to a specific agent/nutrient may provide early markers of disease.[9]

Specific diagnostic probes for proteins using protein array technologies are being developed in the field of proteomics. While genomics identifies the function of genes, proteomics identifies gene products.[10] Proteins are part of physiological and pathophysiological processes in a cell or an organism, and proteomics describes the complete protein inventory.[11] Disease processes affect the protein profile, consequently characterizing protein profiles using protein array technology[12] reveals information for the understanding of disease and possible therapies. This is a coming development in cardiovascular research in which changes in gene expression as a result of nutrient intake changes can now be fingerprinted using these techniques.[13] Analyses at subcellular, and molecular levels have shown subtle intracellular processes associated with coronary heart disease. Proteomic analysis permits fingerprinting at a point in time which can then be compared with a later time after a dietary or pharmaceutical intervention. Protein modifications can then be identified in response to the intervention. Most effort at present is going into the use of this in the development of better and more effective pharmaceutical agents but it has the potential for application in the development of precise markers of nutrient intake in relation to disease outcome. Functional proteomic studies, which identify protein modification together with functional data from established biochemical and physiological methods, will eventually lead to better understanding of the interplay between proteome change and disease outcome.[13]

Conclusion

Screening for biomarkers at the gene or protein expression level using microarray technology has the potential to identify new biomarkers for disease diagnosis. With most of the 30 000 or so human genes sequenced, it has become possible to determine using microarray technology sets of genes that are likely to be involved in a nutrient-dependent biological process. The responses of those genes to the nutritional intervention, will provide highly specific biomarkers of the functions of nutrients. Such nutrient–gene interactions will be of particular interest in explaining the variability in the responses of individuals to a given nutrient. It will also permit better subject selection for intervention trials, providing more effective outcomes in a targeted selection of the population. In this way, genomics and proteomics, by defining the intervention through a particular pattern of protein expression, offer a way of selecting those most likely to benefit (better targeting of at risk groups). Whether this will enable a better understanding of food–gene interactions to permit health claims rather than better therapeutic treatment (at high economic cost) remains to be demonstrated.

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