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Mechanism discovered for pollutant-induced cancer

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Researchers from the Johns Hopkins School of Public Health (Baltimore, MD, USA) have outlined the molecular process where a tumour-promoting toxin (phorbol 12-myristate 13-acetate; PMA) activates an airway squamous cell differentiation marker gene, *SPRR1B* (Ref. 1). *SPRR1B* is one of the building blocks of the cornified envelope, a terminal phenotype of squamous cell differentiation, believed to be a precancerous lesion in tracheobronchial epithelial cells[2].

The findings could have implications for the development of new treatments for lung cancer, the leading cause of cancer death in developed countries. Approximately 80% of patients diagnosed with lung cancer die within 12 months because this type of cancer is asymptomatic in the early stages and usually remains undetected until it has reached an advanced stage.

Environmental pollutants and *SPRR1* induction

The expression of squamous cell function in the respiratory tract epithelium is a phenomenon frequently associated with injury caused by environmental pollutants. Earlier studies by Sekhar Reddy and his colleagues at the University of California at Davis (Sacramento, CA, USA) have demonstrated a close relationship between the induction of the human small proline-rich protein type 1B (*SPRR1B*) gene and squamous cell differentiation in airway epithelium[3,4]. This is supported by another study in which an early upregulation of *SPRR1* gene expression was demonstrated during tobacco smoke-induced squamous metaplasia in rat nasal epithelia[5].

However, it appears that after cancer appears, *SPRR1B* gene expression is turned off and SPRR1B protein production ceases. 'We believe the rise in production and the quick disappearance of the *SPRR1* expression is related to the development of lung cancer,' says Reddy, now an Assistant Professor in the Department of Environmental Health Sciences at the Johns Hopkins School of Public Health.

SPRR genes

SPRR1B belongs to a multigene family (where similar genes are located next to each other) consisting of two *SPRR1* genes (*SPRR1A* and *SPRR1B*), seven *SPRR2* genes (*SPRR2A* to *SPRR2F*) and one *SPRR3* gene[2]. The *SPRR* family of genes encode small MW proteins rich in proline, cysteine and glutamate that are differentially expressed in the suprabasal epithelial layer of various squamous tissues. They crosslink to themselves and cornified envelope precursor proteins, such as loricrin and involucrin, and play an important role in modifying the biochemical properties of squamous tissue. The terminal step of squamous differentiation is the formation of a cornified envelope catalysed by transglutaminase to form an insoluble mesh, which forms a tough extra barrier against exposure to toxins.

'All the SPRR genes have similar functions but show different expression in different squamous tissue. In contrast to squamous tissues such as oesophagus, tongue and skin, which contain higher levels of *SPRR1B* message, the presence of *SPRR1B* message level is very low in respiratory tract epithelia that normally express mucociliary functions,' says Reddy.

SPRR1B genes and the respiratory tract

Recently, immunochemical and transient transfection analysis of various mutants of the *SPRR1B* gene in human tracheobronchial epithelial cells showed the participation of SPRR1B protein in airway epithelial cell cornification induced by PMA (Ref. 6).

In earlier studies, Reddy and his colleagues showed that agents that promote squamous cell differentiation also induce changes in *SPRR1B* gene levels. Furthermore, in airway epithelia, PMA (a toxic component of cigarette smoke) stimulates the expression of the *SPRR1B* gene 5–10-fold[3].

In further experiments, they combined *in vivo* footprinting, deletion and site-directed mutation analysis to map the promoter elements that regulate the gene. They showed that the –152 to +12 base pair promoter region contains two functional activator protein 1 (AP-1) sites that are required for both basal and PMA-enhanced SPRR1B promoter regulation[7].

In the present study[1], Reddy and colleagues explored the signal transduction pathway responsible for PMA producing the SPRRIB protein using *in vitro* cultures

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of human tracheobronchial epithelial cells. To elucidate the pathway, they used both pharmacological agents and dominant negative mutant proteins that specifically inhibit various steps of the cellular signalling pathway. Dominant negative mutant protein, when overexpressed in the cell, specifically inhibits the activity of endogenous protein. For example, they found that the dominant negative protein kinase C δ (PKCδ) and rottlerin (a pharmacological inhibitor of PKCδ) abolished PMA-stimulated *SPRR1B* gene expression. Furthermore, mitogen-activated kinase kinase 1 (MKK1) inhibitors and its mutant protein suppressed PMA-enhanced SPRR1B promoter activity. However, mutants of extracellular signal-regulated kinases 1 and 2, downstream targets of MKK1, did not affect SPRR1B promoter regulation, indicating involvement of a yet unidentified extracellular-signal-related kinase (ERK)-like kinase in PMA-stimulated expression of SPRR1B.

The future

Currently, the team is repeating the process using human lung cancer cell lines to see if they show any defects in the pathways. 'If we can understand these early precancerous cellular changes, we might be able to reverse them before it is too late. Alternatively, if we can find a way to detect pre-cancerous cells within a few months of their onset, we might be able to prevent the development of full-blown cancer,' says Reddy. Further studies include the examination of lung biopsies to identify the stage at which *SPRR1B* gene expression is lost. Loss of gene expression could be developed as a diagnostic tool for lung cancer.

One treatment approach, says Reddy, would be to use cornification as a targeted cancer treatment. 'If you could induce cornification in cancer cells this would kill them and/or consequently slow the growth of the cancer.'

References

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People

Two transcription regulation experts join Science Board

CIStem Molecular Corporation (San Diego, CA, USA) has appointed two leading experts to their Scientific Board. The addition of the transcriptional regulation experts, Keith Tamamoto and James Kadonaga, to the Board is hoped to help in the company's aim to develop technologies for the characterization and commercialization of genetic regulatory circuits that control gene expression.

Yamamoto is currently Professor and Chairman of the Department of Cellular and Molecular Pharmacology at the University of California (San Diego, CA, USA) and has made significant contributions to the knowledge of signalling and gene regulation mechanisms through intracellular receptors that mediate the actions of several essential hormone classes. He is also the outgoing Chairman of the Advisory Committee to the NIH Center for Scientific Review, is a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences.

Kadonaga is currently Professor of Molecular Biology at the University of California and has focussed on transcriptional regulation and chromatin structure. His research has included the development of sequence-specific DNA affinity chromatography, cloning of transcription factor Sp1, discovery of the downstream core promoter element (DPE), the use of chromatin templates to recreate ligand-regulated transcription by nuclear factors *in vitro*, and the cloning of factors that mediate chromatin assembly.

Change in investment adviser and manager for the IBT

The Board of International Biotechnology Trust plc (London, UK) has appointed Schroder Ventures Life Sciences Advisors to be their investment advisers and Schroder Investment Management Ltd to oversee the administration of the company. These appointments follow the termination of the previous contract with Rothschild Asset Management.

The Board has negotiated an annual investment management fee of 1.35% of gross assets. Incentive fee arrangements have also been put in place, which include benchmarks and hurdle rates in line with industry practices. The Board consulted several substantial shareholders before making this appointment and will be publishing a circular outlining certain proposals. These proposals are aimed to enable shareholders who wish to retain their investment in the international biotechnology sector to do so through a vehicle whose initial net value will comprise of not less than 50% of the company's assets.

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