

RUNX and Cancer

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There is a renewed realisation that dysregulation of cell differentiation induces cancer. This notion is partly based on the fact that many of the oncogenes and tumour suppressor genes so far identified are developmental regulators. The runt-related (RUNX) genes are the mammalian homologs of the *Drosophila* segmentation gene, runt.

There are three RUNX genes, RUNX1/AML1, RUNX2/CBFA1 and RUNX3/PEBP2aC, all of which have been shown to have oncogenic potential. The RUNX genes are now known to be involved in the regulation of diverse biological activities. RUNX1 is essential for the generation of hematopoietic stem cells and is most frequently involved in human acute leukaemia (30%), mainly as a target of chromosome translocations. RUNX2 is required for maturation of osteoblast. In the mouse system, the gene is shown to cooperate with c-myc to induce B-cell lymphoma. RUNX3 is most recently characterised and shown to be a tumour suppressor of gastric cancer. In this article, we will first describe how we are exploiting RUNX1 to hunt a large number of cancer causing genes and then describe our results on the involvement of RUNX3 in gastric cancer.

The past several decades of research have uncovered a number of oncogenes and tumour suppressor genes but the current list of these genes by itself does not explain how these genes are involved in multi-step carcinogenesis. On the one hand as we still continue searching for as yet unknown key cancer-causing genes we must, at the same time, devise an experimental system to analyse the relationship among these genes. These studies are important not only for the better understanding of carcinogenesis but also for creating a foundation for future drug discoveries. A powerful approach has been described for this purpose based on a mouse leukaemia model using retroviruses. This method relies on the property of a strain of retrovirus which integrates host cell DNA randomly and either activates oncogenes or destroys tumour suppressor genes to cause leukaemia. The best example is the BXH2 leukaemia-prone mouse. The BXH2 mouse contains an endogenous retrovirus which is released in the body and integrates randomly into host chromosomes. As a result of this, the mouse develops acute myeloid leukaemia at 10 to 12 months of age in 90% of all cases.

The viral integrations can be used as tags to easily fish out cancer associated genes by the inverse polymerase chain reaction (IPCR). Typically, three genes are affected by the retrovirus in this mouse model. Therefore, the BXH2

leukaemia-prone mouse strain is a high throughput method to detect cancer causing genes.¹ Although many (~50~100) potential cancer-causing genes have been identified by this method, functional interactions among the genes are not known. Therefore, putting all these genes in perspective to understand the grand view of carcinogenic processes is the subject of the next generation of research.

RUNX1 is the most frequent target of chromosome translocations in acute leukaemia. We found that haploinsufficiency of RUNX1 is also leukemogenic.² In fact, familial cases are known in which one allele of RUNX1 is mutated and family members frequently develop acute myeloid leukaemia (familial platelet disorder with propensity to acute myeloid leukaemia).³ Based on these observations, we attempted to generate a mouse model of FPD-AML and knocked out one of the alleles of Runx1 of BXH2 mouse to observe the leukemogenic process. In other words, we introduced one genetic change at the beginning of the experiment. As expected, we observed the development of leukaemia in BXH2 (Runx1+/-) mice two months earlier than the parental BXH2 mice, suggesting strongly that haploinsufficiency of Runx1 is leukemogenic also in the mouse and that the second and third alterations of genes which must have occurred in these mice cooperate with Runx1 (+/-). We expect that by identifying the altered genes, we will accumulate the genes which functionally cooperate with inactivated Runx1. The results will be critical in understanding the functional cooperation among cancer causing genes. Since RUNX1 is responsible for approximately 30% of all types of human leukaemia, the genes altered in Runx1+/- BXH2 (Runx1+/-) should be very valuable in the study of human leukemogenesis.

As multiple genetic changes, at least three in leukaemia, are required for the development of cancer, abnormality of only Runx1 is not sufficient to give rise to clinically overt leukaemia. If one of these additional hits is controlled adequately by a small molecule, there is a possibility that we will see a remission of the cancer in leukaemia patients.

We already have approximately 65 DNA samples from BXH2 leukaemias. Each leukaemia sample in BXH2(Runx1+/-) usually contains three to five genetic hits (three to five retrovirus integrations in BXH2 mice).

The molecular mechanisms by which epithelial cells of gastric mucosa are converted to cancer cells are poorly understood. One of the main reasons is that the genes involved in normal development and differentiation of stomach epithelial

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cells, and those involved in the carcinogenic processes of cells are largely unknown.

We recently reported that the loss of expression of the runt-related gene 3 (RUNX3) is causally related to gastric cancer.⁴ RUNX3 is a member of the three gene family encoding the DNA binding subunit of the Runt domain transcription factor, PEBP2/CBF.⁵ In the mouse, Runx3 is ubiquitously expressed. In the stomach of the adult mouse, Runx3 is expressed most prominently in pepsinogen-producing chief cells and surface epithelial cells, both of which are terminally differentiated in gastric mucosa.

In Runx3^{-/-} mice, hyperplasia of gastric mucosa is observed due to insensitivity of the epithelial cells to TGF- β mediated growth inhibition and TGF- β induced apoptosis.⁴ 15 cell lines isolated from human gastric cancer⁷ showed no or reduced expression of RUNX3 due to hemizygous deletion and hypermethylation of the promoter region. In 46 surgically resected gastric cancer specimens, 9 out of 22 stage I (41%), 1 out of 2 stage II (50%), 11 out of 14 stage III (79%) and 7 out of 8 stage IV (88%) cases exhibited reduction of RUNX3 expression, respectively. Then we looked for mutations in RUNX3 in gastric cancer specimens and found one, R122C, in which arginine 122 was replaced by cystine after screening of more than 150 specimens. The mutation was located within the conserved Runt domain required for the DNA binding and heterodimerization with the β subunit.⁴

To test whether RUNX3 has a tumour suppressor activity, a cell line, MKN28 which does not express RUNX3, was inoculated subcutaneously in nude mice and found to be tumourigenic. Two independent MKN28 cell lines stably expressing exogenous RUNX3 were poorly tumourigenic. When two independent stable lines expressing the RUNX3 (R122C) were inoculated, no tumour suppressor activity was detected. These results strongly suggested that RUNX3 is a tumour suppressor of gastric cancer.

It is known that the silenced genes due to hypermethylation of the promoter region could be reactivated by treating cells with inhibitors of methyltransferase such as 5'-aza-cytidine and histone deacetylase, such as trichostatin A.⁶ We treated three gastric cancer cell lines which do not express RUNX3 with these inhibitors alone or in combinations and found that RUNX3 was indeed reactivated by either one of these inhibitors.

The results confirmed that the gene silencing is due to DNA methylation of the promoter region and recruitment of histone deacetylase near the promoter region.

As mentioned above, mutations in RUNX3 are very rare. Therefore, when the gene is reactivated in gastric cancer cells, the gene is, in most of the cases, free of any mutation. Therefore, there is a high hope that tumour growth in gastric cancer may potentially be controlled by inducing the expression of silenced RUNX3 using reagents such as histone deacetylase inhibitors. The mouse model of gastric cancer to test this possibility is being established.⁷

RUNX3 is on 1p36 where it has been predicted that an important tumour suppressor is located, since LOH of this locus is observed at high frequency not only in gastric cancer but also in many gastrointestinal and other cancers. RUNX3 may be this long sought-after tumour suppressor and, at the same time, the product of the gene may be the primary target of TGF- β -mediated tumour suppressor pathway. If these predictions are correct, RUNX3 would be involved in many different types of cancers.⁸

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