

IISER Bhopal: strategies to fight breast cancer

The first study of DNA methylation causing alternative splicing in cancer cells

R. PRASAD

Inhibiting the growth and accelerating the death rate of breast cancer cells may be possible by starving the cancer cells of glucose or by inhibiting the energy production process (aerobic glycolysis). Aerobic glycolysis confers a proliferative advantage to the cancer cells. The results were published in the *Proceedings of the National Academy of Sciences*.

A team of researchers led by Dr. Sanjeev Shukla from the Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Bhopal, has been able to reverse aerobic glycolysis by inhibiting DNA methylation or reducing the expression of BORIS (Brother of Regulator of Imprinted Sites) gene.

Alternative splicing

In normal cells, exon 9 gets included while exon 10 is excluded when precursor messenger RNA (pre-mRNA) is spliced or edited into mature messenger RNA (mRNA). The researchers found that splicing was different in the case of cancer cells – instead of exon 9,



Positive: "Targeting BORIS will have a great impact for cancer therapy," say Smriti Singh (seated) and Sanjeev Shukla ■

exon 10 gets included when the pre-mRNA is spliced into mRNA. "The methylation at exon 10 allows BORIS to bind to the exon 10 and leads to the inclusion of exon 10 in the mature mRNA," says Dr. Shukla. "Due to aberrant alternative splicing, exon 10 gets included in the mature mRNA leading to the formation of PKM2, a cancer-specific isoform of pyruvate kinase."

The PKM2 isoform is seen in cancer cells and is responsible for proliferation of cancer due to aerobic glycolysis while the PKM1 iso-

form is found in normal cells. The DNA methylation along with BORIS regulates the switch from PKM1 to PKM2 isoform.

This is the first time DNA methylation causing alternative splicing in cancer cells has been studied. DNA methylation also silences tumour suppressor genes.

"The BORIS gene is predominantly expressed in germ cells and gets over-expressed in cancer cells but not in somatic cells. So BORIS is a potential target for cancer therapy," says Dr. Shukla.

The team studied two breast cancer cell lines and found increased exon 10 in both cancer cell lines but exon 9 was found to be increased in normal, primary cells. The presence of exon 10 in breast cancer cell lines led to higher expression of cancer-specific PKM2 isoform.

Unlike in normal breast tissue, the researchers found overexpression of BORIS in breast cancer subtypes in the Cancer Genome Atlas and Oncomine.

Potential targets

Inhibition of DNA methylation led to reduced binding of BORIS at exon 10 leading to reduced exon 10 and increased exon 9 in the mature mRNA. There was also a reduction in the cancer-specific PKM2 isoform and increase in the PKM1 isoform.

Similarly, depletion of BORIS gene led to reduced BORIS binding and decreased inclusion of exon 10 with a concomitant increase in exon 9 inclusion.

To rule out other factors responsible for alternative splicing of PKM, the researchers mutated the BORIS binding site at the

PKM exon 10 region. "There was reduced exon 10 and increased exon 9 in the mutant cells. Also, there was reduced BORIS enrichment which resulted in reduced exon 10 inclusion in the mutant cells," Dr. Shukla says.

As predicted, BORIS depletion reduced the consumption of glucose in breast cancer cells. Inhibition of DNA methylation, too, decreased glucose uptake. Interestingly, BORIS depletion went beyond reduction in glucose consumption – it resulted in reduced cancer cell growth and cell viability.

"When BORIS is down regulated it affects alternative splicing of several genes which are associated with cancer hallmark (cancer growth, reduced apoptosis abnormal metabolism). So targeting BORIS will have a great impact for cancer therapy," says Smriti Singh from the Department of Biological Sciences, IISER, Bhopal, and the first author of the paper. "BORIS binds after DNA methylation. So we must determine whether inhibiting DNA methylation or BORIS is more effective in cancer therapy."