news

The Nobel Prize in Physiology or Medicine for 2006

On October 2, 2006, the Nobel Assembly at Karolinska Institutet had decided to award The Nobel Prize in Physiology or Medicine for 2006 jointly to Andrew Z. Fire (at the Stanford University in California, USA) and Craig C. Mello (at the University of Massachusetts in Worcester, USA) for their discovery of "RNA interference – gene silencing by double-stranded RNA".

Andrew Z. Fire

Craig C. Mello

This year's Nobel Laureates have discovered a fundamental mechanism for controlling the flow of genetic information. The human genome operates by sending instructions for the manufacturing of proteins from DNA in the nucleus of the cell to the protein synthesizing machinery in the cytoplasm. These instructions are conveyed by messenger RNA (mRNA). In 1998, the favoured scientists published their discovery of a mechanism that can degrade mRNA from a specific gene (Fire A, Xu SQ, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998;391:806-11). This mechanism, RNA interference, is activated when RNA molecules occur as double-stranded pairs in the cell. Double-stranded RNA activates biochemical machinery, which degrades those mRNA molecules that carry a genetic code identical to that of the double-stranded RNA. When such mRNA molecules disappear, the corresponding gene is silenced and no protein of the encoded type is made.

Post-transcriptional gene silencing, initially considered a bizarre phenomenon limited to petunias and a few other plant species, is now one of the hottest topics in molecular biology. Around 1990, molecular biologists obtained a number of unexpected results that were difficult to explain. The most striking effects were observed by plant biologists who were trying to increase the colour intensity of the petals in petunias by introducing a gene inducing the formation of red pigment in the flowers. Instead of intensifying the colour, this treatment led to a complete loss of colour and the petals turned white. The mechanism causing these effects remained enigmatic until Fire and Mello made the discovery for which they have received this year's Nobel Prize. They were investigating how gene expression is regulated in the nematode worm Caenorhabditis elegans. Injecting mRNA molecules, which encode a muscle protein, led to no changes in the behaviour of the worms. The genetic code in mRNA is described as being the 'sense' sequence, and injecting 'antisense' RNA, which can pair with the mRNA, also had no effect. However, when Fire and Mello injected sense and antisense RNA together, they observed that the worms displayed peculiar, twitching movements. Similar movements were seen in worms that completely lacked a functioning gene for the muscle protein. They proposed that RNA interference is a catalytic process. Double-stranded RNA binds to a protein complex, Dicer, which cleaves it into fragments. Another protein complex, RISC, binds these fragments. One of the RNA strands is eliminated but the other remains bound to the RISC complex and serves as a probe to detect mRNA molecules. When an mRNA molecule can pair with the RNA fragment on RISC, it is bound to the RISC complex, cleaved and degraded. The gene served by this particular mRNA has been silenced. The RNA interference can spread between cells and it can even be inherited. Their discovery clarified many confusing and contradictory experimental observations and revealed a natural mechanism for controlling the flow of genetic information. RNA interference occurs in plants, animals, and humans. Fire and Mello in their studies on gene regulation on the nematode Caenorhabditis elegans have made important contributions to describing and elucidating mechanisms of gene silencing, by which scientists can "knock down" the expression of specific genes. It is of great importance for the regulation of gene expression, participates in defence against viral infections, and transposons, and keeps jumping genes under control. In the future, RNA interference is hoped, to be used in many disciplines including agriculture and clinical medicine and it may open a new avenue for disease treatment.

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High prevalence of isolated tumor cells in regional lymph nodes from pN0 colorectal cancer

Like other solid tumors, colorectal cancer is pathologically staged based on the extent of primary organ involvement and the metastatic spread to lymph nodes or to distant organs. Metastasis in regional lymph nodes is one of the most important factors relating to the prognosis of colorectal carcinoma and discriminates patients requiring postsurgical adjuvant treatments. Although patients with no extranodal metastasis, in the stages 0, I, and II of colorectal cancers are regarded as having localized disease, as many as 35% of patients without lymphonodal metastatic tumors deposit, develop extranodal metastases within 5 years after surgery and eventually die from colon cancer. The early identification of this subgroup of patients would allow postsurgical therapeutic measures, possibly resulting in a lower rate of cancer recurrence.

A possible explanation and a partial cause of this phenomenon may result from pathological understaging of the tumor, by the failure to identify lymphonodal metastatic when they are present. Missing positive nodes will understage patients and cause adjuvant therapy to be withheld from a group of patients that could benefit from it. More scrupulous examination of lymph node within a resected specimen could improve the detection of node-positive disease. Examination of a minimum of 12 lymph nodes per colon cancer specimen was adopted by the American Joint Committee on Cancer and the TNM Committee of the International Union Against Cancer. It is not a new finding that a more detailed microscopic assessment of lymph nodes (serial or step sectioning) or a more sensitive method of detec-

tion of microscopic involvement (immunohistochemistry) of lymph nodes can reveal tumor cells undetected by standard hematoxylin and eosin examination.

In the spectrum of lymph node colonization by cancer cells, three main situations occur: 1) metastases (metastatic implants with a diameter >0.2 cm); 2) micrometastases (macroscopically undetectable metastases ranging between 0.02 and 0.2 cm in diameter); and 3)isolated tumor cells (which are single or small nests of countable tumor cells, with a diameter never >0.02 cm, only detectable by immunohistochemistry or molecular biology methods). The current nomenclature suggests that the presence of isolated tumor cells in lymph nodes should be reported as pN0(i+) or pN0(mol+), where "i" and "mol" indicate the methods used for isolated tumor cells detection (immunohistochemistry and molecular methods, respectively). In patients with colorectal carcinoma, the prevalence and clinical effect of lymph node micrometastases and utility of immunohistochemistry remain controversial.

A group of scientists from Italy, study group included 309 colorectal cancer patients without lymph node metastases or micrometastases were detected by conventional histological examination. From the total of 5016 lymph nodes (average number of lymph node 16,23), two additional histological sections (10 032 sections, in all) 75–100 μm apart were obtained and stained with monoclonal anti-cytokeratin MNF116 antibody (clone MNF116 mouse monoclonal antibody; Dako, broad spectrum anti-keratin antibody, reacting with intermediate and low-molecular-weight keratins, cytokeratins 5,6,8,17,19).

Isolated tumor cells were defined as phenotypically malignant (i.e. altered nucleus to cytoplasm ratio, atypical nuclei and prominent nucleoli), unequivocally MNF116-positive single cells dispersed in sinusoidal or extra-sinusoidal spaces. Clustered isolated tumor cells never consisted of more than 2–8 cells and never exceeded 0.02 cm in diameter. Isolated tumor cells were identified in 1 of 25 (4%) patients with pTis, 4 of 28 (14.3%) patients with pT1, 44 of 95 (60%) patients with pT2, 100 of 150 (72.7%) patients with pT3 and 7 of 11 (63.7%) patients with pT4 cancer. This study of patients with pN0M0 colorectal cancer, the prevalence of pN0(i+) cases was 50.5%, the highest percentage reported in the literature. A significant correlation was detected between isolated tumor cells status and pT value. Concordant isolated tumor cells picture was seen in the two histological sections in 4730 of 5016 (94.3%) lymph nodes (4520 lymph nodes without isolated tumor cells and 210 concordant isolated tumor cells positive lymph node sections). In 286 of 5016 (5.7%) lymph nodes, only one section from the same lymph node showed MNF116+ cells (discordant lymph node sections). A significant association was found between vascular invasion and the number of isolated tumor cells positive lymph nodes. Statistically significantly highest number of lymph node with isolated tumor cells were found only in lymph nodes located within 3 cm from the neoplasia.

The growth of nodal metastases results from a multistep process, which includes the arrest, extravasation, implantation and proliferation of tumor cells. The significant relationship between lymph node with presence of isolated tumor cells, cancer p-TNM stage and vascular invasion implies a clinicobiological relevance of isolated tumor cells and indicates that efforts should be made to prospectively evaluate the prognostic significance of this lesion. Further studies are required to prospectively evaluate the clinicobiological relevance and the prognostic significance of isolated tumor cells. Special attention should be paid in colorectal cancer staging to accuracy retrieving the lymph nodes nearest to the cancer site. The presence of isolated tumor cells in regional lymph nodes might help to explain pN0 early-relapsing colorectal cancers.

REFERENCE

1. Mescoli C, Rugge M, Pucciarelli S, Russo VM, Pennelli G, Guido M, Nitti D. High prevalence of isolated tumor cells in regional lymph nodes from pN0 colorectal cancer. J Clin Pathol 2006;59:870-4.

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Sentinel-node biopsy or nodal observation in melanoma

The staging of intermediate-thickness (1.2 to 3.5 mm) primary melanomas according to the results of sentinel-node biopsy provides important prognostic information and identifies patients with nodal metastases whose survival can be prolonged by immediate lymphadenectomy. (ClinicalTrials.gov number, NCT00275496).

In most patients with clinically localized melanoma of intermediate thickness, wide resection is curative, but metastasis to regional nodes develops in 15 to 20%. Since metastasis to a regional node is the most important prognostic factor in early-stage melanoma, immediate (elective) lymphadenectomy has been advocated to improve tumor staging and possibly survival. However, this approach exposes patients to complications resulting from the procedure and has not been shown to improve overall survival; in a minority of patients with occult nodal metastases, however, it may have benefit.

The Multicenter Selective Lymphadenectomy Trial (MSLT) was initiated on January 4, 1994, to study the usefulness of sentinel-node biopsy in the identification of patients with clinically occult nodal metastases and to evaluate the clinical effect of immediate, complete lymphadenectomy in patients with tumor-positive sentinel nodes. Enrollment in the trial closed in March 2002. After the third planned interim analysis, the data and safety monitoring committee recommended publication of data with implications for the management of early-stage melanoma. The data on surgical complications and the accuracy of sentinel-node biopsy have been published elsewhere; this report presents interim data on the efficacy end points of the trial.

This interim analysis did not reveal a significant difference in melanoma-specific survival between the two study groups, but it did show that biopsy with immediate lymphadenectomy prolonged disease-free survival and diminished the trauma of recurrence.Observation allows nodal micrometastases to enlarge and spread to other nodes, thereby increasing the risk of distant metastases and decreasing the chance of long-term survival (Figure 3B). Immediate lymphadenectomy in patients with subclinical sentinel-node metastases increased the melanoma-specific 5-year survival rate, as compared with delayed lymphadenectomy for clinically detected nodal relapse (72.3% vs. 52.4%; hazard ratio for death, 0.51; 95% CI, 0.32 to 0.81; P=0.004) (Figure 3B). We did not expect that the removal of tumor-free regional nodes would improve survival — indeed, biopsy did not improve survival among patients without nodal metastases (Figure 3C).

Our results provide evidence that occult micrometastases in the sentinel node usually progress to aggressive regional or distant disease. Were this not the case, we would not have seen an overall improvement in disease-free survival among the patients assigned to biopsy nor would there have been a significant difference in the rate of nodal relapse between patients with tumor-negative sentinel nodes and those assigned to observation (4.0% [26 of 642 patients] vs. 15.6% [78 of 500], P<0.001) (Figure 3A). The influence of the tumor status of the sentinel node on disease-free survival and melanoma-specific survival (P<0.001 for both comparisons) also indicates the aggressiveness of sentinel-node micrometastases.

Panel A shows the time to clinical nodal recurrence in the observation group and to an initial nodal recurrence after a false negative result on sentinel-node biopsy. Panel B shows the melanoma-specific survival among patients with nodal metastases: subgroup 1 comprised patients with a tumor-positive sentinel node; subgroup 2, the patients in subgroup 1 plus those in subgroup 4 with a nodal recurrence after a negative result on biopsy; subgroup 3, those with nodal recurrence during observation; and subgroup 4, those with nodal recurrence after a negative result on biopsy. Panel C shows the melanoma-specific survival among patients without nodal metastases, according to the type of treatment (median follow-up, 59.8 months). Panel D shows the time to local or in-transit metastasis, according to the type of treatment.

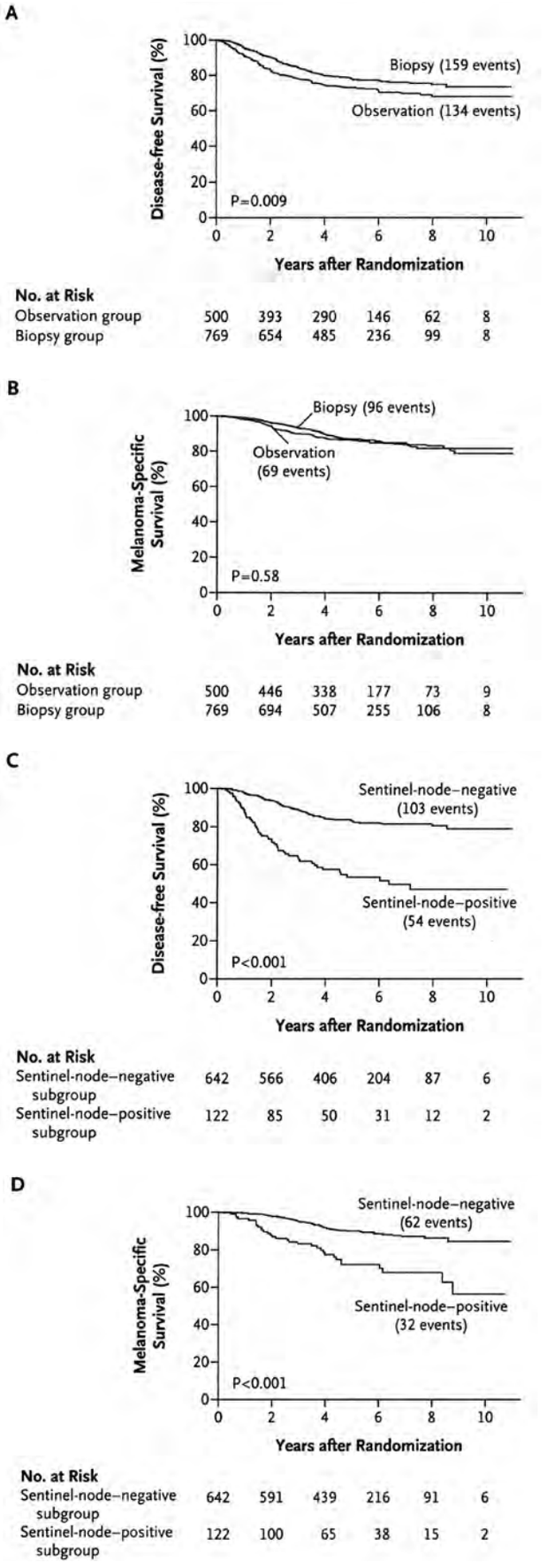

Figure 3. Melanoma-Specific Survival, According to the Presence or Absence of Nodal Metastases and Time to Nodal and Local or In-Transit Recurrence. (Taken from ref. 1)

REFERENCE

1. Morton DL, Thompson JF, Cochran AJ, Mozzollo N, Elashoff R, Essner R, et al. Sentinel-Node Biopsy or Nodal Observation in Melanoma. N Engl J Med 2006;355(13):1307-17.
2. Morton DL, Cochran AJ, Thompson JF, et al. Sentinel node biopsy for early-stage melanoma: accuracy and morbidity in MSLT-I, an international multicenter trial. Ann Surg 2005;242:302-11.
3. van Poll D, Thompson JF, Colman MH, et al. A sentinel node biopsy does not increase the incidence of in-transit metastasis in patients with primary cutaneous melanoma. Ann Surg Oncol 2005;12:597-608.
4. Kang JC, Wanek LA, Essner R, Faries MB, Foshag LJ, Morton DL. Sentinel lymphadenectomy does not increase the incidence of in-transit metastases in primary melanoma. J Clin Oncol 2005;23:4764-70.

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Transmission of human herpesvirus 8 by blood transfusion

Kaposi's sarcoma is the most common cancer associated with the acquired immunodeficiency syndrome (AIDS) worldwide, and human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma–associated herpesvirus, was identified a decade ago as the causative agent of Kaposi's sarcoma. The burden of Kaposi's sarcoma in Africa is high; in Uganda, Kaposi's sarcoma accounts for half of all reported cancers. In industrialized countries, the seroprevalence of HHV-8 is relatively low (2 to 8%), whereas in sub-Saharan Africa, the seroprevalence of HHV-8 can exceed 50%. The modes of transmission of HHV-8 in Africa remain poorly understood. Studies indicate that the seroprevalence increases throughout childhood and reaches a plateau by adolescence, suggesting that transmission occurs mainly in the community, probably through saliva or other nonsexual routes.

Whether HHV-8 is transmitted by blood transfusion remains controversial. Transmissibility of the virus by this route may be limited by the cell-associated nature of the virus and the low frequency of circulating virus in asymptomatic seropositive persons. Previous studies that did not find evidence of transfusion-transmitted infection enrolled small numbers of patients, most of whom received leukocyte-reduced or acellular blood components.

The potential for blood-borne transmission of HHV-8 has been supported by the results of a number of studies.

To evaluate the risk of the transmission of HHV-8 by blood transfusion, we conducted a prospective observational cohort study of transfusion recipients in Uganda, where the seroprevalence among blood donors was 40%,leukocyte reduction was not used, and blood storage time was usually short. If transmission of HHV-8 by transfusion occurs, it is likely to be detected in such a setting.

Patients who had received a transfusion of any HHV-8–seropositive blood products were categorized as exposed, regardless of the serologic status of additional units. Patients who had received transfusions of HHV-8–seronegative blood alone were categorized as unexposed. During the entire 6-month follow-up period, there were 24 seroconversions among exposed recipients, as compared with 17 among unexposed recipients ($P<0.05$) (Panel A). During the first 3 to 10 weeks after transfusion, there were 14 seroconversions among exposed recipients, as compared with 4 among unexposed recipients ($P=0.005$) (Panel B).

We conducted a prospective cohort study assessing the risk of transfusion-associated HHV-8 infection in a large population of linked blood donors and transfusion recipients. Patients who received HHV-8–seropositive blood were significantly more likely to become infected than were recipients of seronegative blood. The increased risk associated with receiving HHV-8–seropositive blood was most striking among recipients in whom serocon-

version occurred 3 to 10 weeks after transfusion, an interval that is similar to the timing of the immune response for other transfusion-transmitted herpesviruses. The risk of seroconversion was also higher among recipients of seropositive units that had been stored with shorter storage times than among recipients of blood that had been stored for more than 4 days (excess risk, 4.2%), as has been found with other herpesviruses. Together, these results provide compelling evidence of the transmission of HHV-8 by blood transfusion.

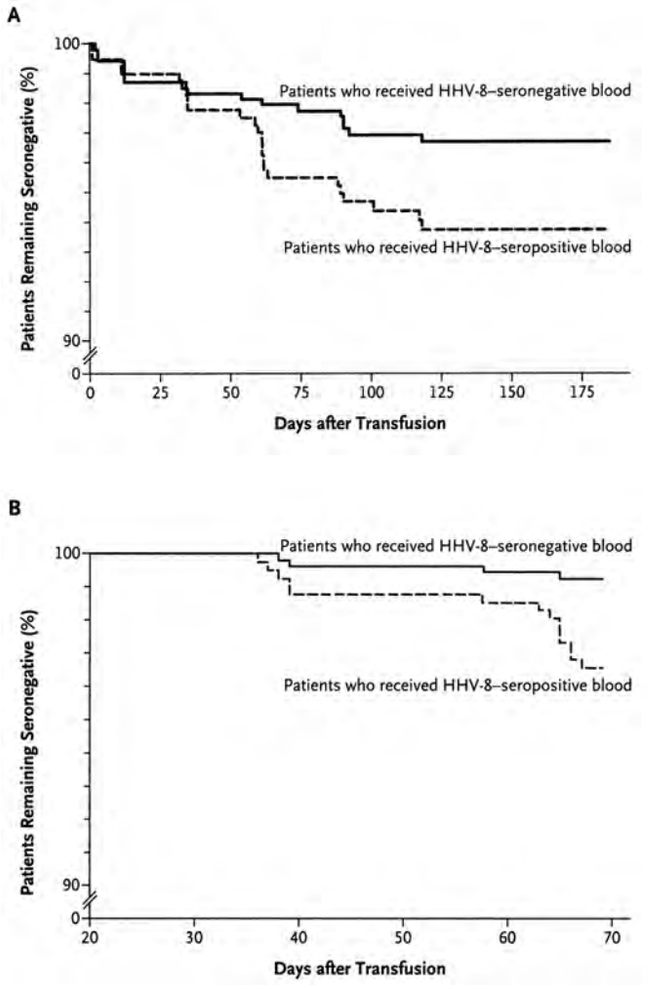

Figure 2. Kaplan–Meier Analysis of the Percentage of Transfusion Recipients Who Remained Seronegative for the Entire 6-Month Follow-up Period (Panel A) and from Week 3 to 10 after Transfusion (Panel B), According to Whether They Were Exposed to HHV-8–Seronegative or HHV-8–Seropositive Blood. (Taken from ref. 1)

REFERENCE

1. Transmission of Human Herpesvirus 8 by Blood Transfusion. Hladik W, Dollard SC, Mermin J, Fowlkes AL, Downing R, Amin MM, Banage F, et al. N Engl J Med 2006;355(13):1331-8.

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