I. GENETICS

CONTRIBUTIONS OF THE MOLECULAR CYTOGENETICS TO THE MANAGEMENT OF THE CHILDHOOD ALL

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Abstract

The study of leukemia is a very present theme and it offers interest to many international research groups. In the last years remarkable progresses were made in the treatment of these affections. The accomplishments made in the children acute lymphoblastic leukemia treatment (ALL) are major aspects of the progress and the efficiency of the modern medical science in collaboration with medical genetics. One of the apropriate genetic techniques and very helpful for detecting ALL is the *in situ* hybridization (FISH). ALL is accepted nowadays as an exceptional case of curable cancer through a relatively cheap costs chemotherapy. It is considered as an stimulating example for obtaining same results in other cancer affections in children or adults. Despite the promising results, ALL still remains a heavy duty for the medical society around the world. It's been estimated that every year 50,000 new children ALL cases appear, aproximately 40,000 are in poor or insuficient apropriate medical support countries. In consequence, the curring rate is over 80% in rich countries but unfortunately it globaly drops under 50%. The poor global results are not because the incapacity to defeat the abnormal leukemial behaviour but especially because of the unaccesability or wrong utilisaton of the nowadays' therapeutics.

Keywords: chromosomal rearrangements, ALL, FISH

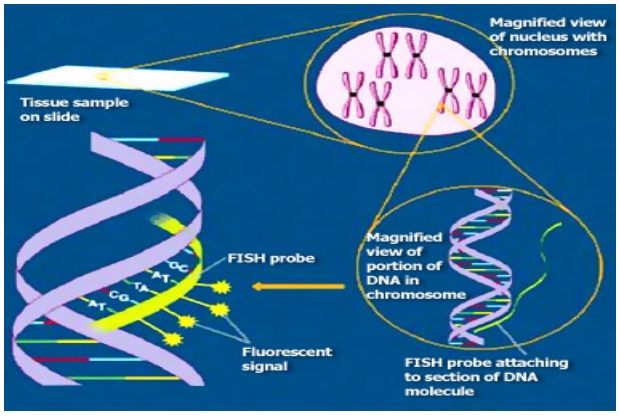
The detection of different nucleic acid sequences of the human chromosomes using the fluorescence *in situ* hybridization is providing the microscopic visualization of the region, making possible for the specialist to see wether there are rearrangements in that area or not. The method uses the fact of specific annealing of complemetary nucleic acid molecules through hydrogen bands between bases attached to the sugar-phosphate backbone as follows :

- adenine (A) anneals with thymine (T, in DNA) or uracil (U, in RNA)
- cytosine (C) anneals with guanine (G)

In this way, the base-pairing leads to the formation of the double-stranded DNA complex, in which the strands have opposite directions one to the other. In conclusion, any nucleic acid sequence can therefore be specifically detected by using the 'antisense' (complementary) sequence.

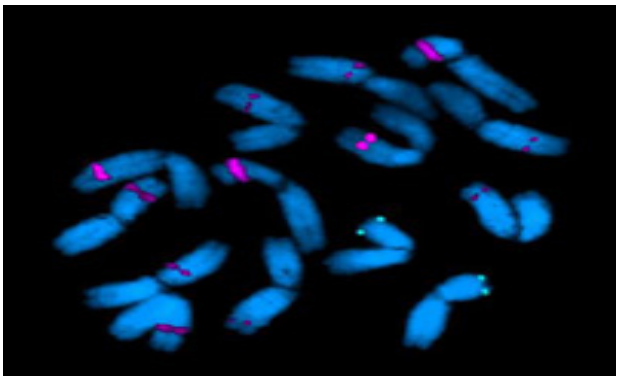
The main steps of the FISH protocol are :

- synthesis of a labelled antisense probe
- pretreatement of slides for increasing the accessibility of target and/or block non-specific sites

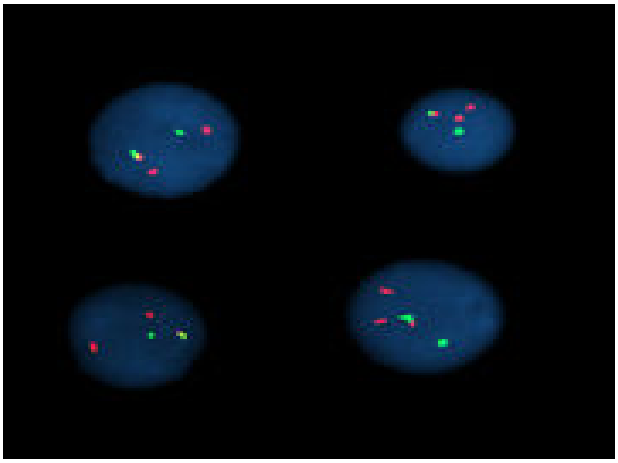


- denaturation of the double-stranded targets
- hybridization detecting fluorescence

Probes for localization by FISH are usually labelled with either biotin or digoxigenin. Deoxyribonucleotides are now also available conjugated to fluorochromes : FITC (fluorescein isothiocyanate), TRITC (tatramethylrhodamine isothiocyanate, and AMCA (aminomethyl coumarin acetic acid). The optimal size of labelled probe fragments is 300 bp. For detecting the labelled probe it is needed a fluorescence microscope with suitable fluorescence objectives and filter sets.



For example, a method of simultaneous hybridization and detection of 2 probes can be achieved using one probe labelled with biotin (detected with Texas red gives a red signal) and the second probe with digoxigenin (detected with FITC is giving a green signal).



Despite these scientifical and technological progresses which concretized in medical successes, ALL still represents a challange for medicine. Still a lot of pacients are dying today in the world and lots of the survivors have all kind of physical and psycho-social marks. Nowadays it is known that the access to the treatment and diagnosis resources must represent a priority to everyone involved in the sanitary sistem and the chance to be cured must be a basic right for all the children in the world. All these are justifying the interest of the medical and scientific society for ALL in the purpose of a better and more precise identification of the biological bases of the treatment resistance and the risk of falling again or the therapy abortion.

The prognosis of ALL in children has changed positively in the last four decades, owing to the conjugated efforts of diverse study groups that used standard criteria for diagnosis and treatment, and which has also permitted result analysis in a short period of time, with the modifications in therapeutical protocols absed on new dicoveries in the leukemic malignant celluar biology. The current treatment is based on a better adaptation of the risk grades and the introduction of medications that acts on the target molecule (eg Introduction of imatinib-mesylatului in ALL BCR-ABL)

The german study group for ALL (BFM) recognized as having a significant result in the treatment of ALL in children, initially uses a risk group inclusion based on clinical criteria, hematological, immunophenotypic, cytogenetic, molecular biology and the response to treatment. According to these criteria, there are three groups:

- low risk : with the leucocyte count <20.000/mmc at diagnosis, age 2-6 yrs, immunophenotype with precursor B, absence of t(9;22)(BCR-ABL) or t(4;11)(MLL-AF4), favorable reponse ro cortizone in day+ 8 of treatment, complete morphologic remission at day +33, residual minimal disease negative in day +33 and +78 evaluated with a high sensibility technique. (10^{-5}/10^{-6})
- intermediate risk : favorable reponse ro cortizone in day+ 8 of treatment, complete morphologic remission at day +33, absence of t(9;22)(BCR-ABL) or t(4;11)(MLL-AF4
- high risk: leukocyte count increased at diagnosis (>100.000/mmc, age > 10 yrs, presence of t(9;22)(BCR-ABL) or t(4;11)(MLL-AF4), non responsive to cortisone treatment at day +8 , absence of complete remission in day +33, minimal residual disease >> 10^{-3} in day +78.

Patients with high risk have indications for intense therapeutical programs which includes even hematopoietic stem cell transplant- a procedure which is now available even in Romania.

The German study group CCG currently identifies another category of children with "very low" risk : female sex, white race, age 1-9 yr, immunophenotype with precursor B cells, hyperploids, leukocytes < 50000/mmc, without CNS involvement at diagnosis, with early response to the treatment (day 8, day14), minimal residual disease < 10^{-2} day +28; for which they propose a less intense chemotherapy- Children's Leukemia & Cancer Research Foundation (Inc), Children's Cancer Group (CCG).

In spite of all these progress in the understanding of the characteristics of the malignant cells, some patients are still over treated and some are under treated. The approach to the leucomogenesis process, which is no more considered secondary to a certain translocation but as a result of complex modifications at the genic level, has changed with the introduction of the genic expression profile analysis.

Using this technology we can analyse over 40.000 genes. We identified 6 risk subgroups of LAL named after criteria mentioned above – LAL-T, E2A-PBX1, TEL-AML1, BCR-ABL, MILL, hyperdiplodia. All of these categories of AL are associated with the abnormal expression of a very large number of genes, their functions in the biology of a normal cell or malign cells is more or less known. This type of studies has evolved during the last 5 years, the first results being published at the end of 2003. In this context, our studies are being aliened with the present preoccupations of the International Science Community.

Nowadays a large number of research groups are studying the AL in children based on the BMR study: European BIOMED-1 Concerted Action "Investigation of minimal residual disease in acute leukemia: international standardization and clinical evaluation" with the participation of 14 diferent laboratories from 8 European countries (ES, NL, PT, IT, DE, FR, SE and AT). Another study undertaken by European Study Group on MRD Detection in ALL (ESG-MRD-ALL)" include 23 laboratories from 10 different European countries (NL, DE, FR, GB, AT, IT, ES, SE, DK, and CZ). Intercontinental-BFM 2002 Protocol (ALL IC BFM 2002) is a larger project produced by American researchers, including BFM group (ALL BFM/AIEOP 2000, Germany, Austria, Italy and Holand), laboratories from Argentina, Chile, Croatia, the Czeck Republic, Hong Kong, Hungary, Israel, Poland si Uruguay, all of them bein intrested in the colaboration with laboratories

world wide. Some of the reports writen by these groups were published in well known magazines, like Blood, Ann Hematol, Lancet, Leukemia, Br J Haematol,. Best Pract Res Clin Haematol,. J Clin Oncol. Klin Padiatr., Nature, Science, New England J Med, Hematology.

In conclusion, the complex diagnostic – morfological, imunophenotipical, citogenetic and molecular – is an obligation in all the international protocols refering to the treatment of ALL sufering patients.

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