Evaluarea prin qPCR a diferentierii sensibilității la terapie și prognosticului in tumorile endocrine (RENET)

Real Time quantitative PCR evaluation of differentiation, drug sensitivity and prognosis of endocrine tumors

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Contractor:
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Abstract

Neuro-endocrine Tumors (NETs) are frequent in the lung (20% of tumors), while uncommon in the Gastro-entero-pancreatic (GEP) system. Their clinical behavior is highly variable, from indolent to highly malignant. Diagnosis and prognostic prediction (hence the therapeutic approach) of NETs is based both on histological criteria and on immunohistochemical (IHC) procedures on formalin-fixed paraffin embedded (FFPE) tissues which, generally, are the only diagnostic material available. A battery of appropriate antibodies is currently employed in order to detect biological markers. The project proposes to substitute the battery of IHC tests with a Real Time quantitative PCR (RT-qPCR) analysis detecting the mRNA specific for the above markers. The project stems from the recent technical evolution permitting RT-qPCR tests on RNA extracted from FFPE tissue blocks. The advantages offered by this approach are that the data are quantitative (unlike the IHC tests) and that a single reaction will permit the analysis of several genes of interest. This novel approach is economically sound since customized arrays are offered by commercial companies at a cost similar to that of the battery of IHC tests. The RT-qPCR approach, whose application to the study of NETs is fully original, will allow to define differentiation status (type of hormone produced), proliferative activity, presence of somatostatin receptors of NETs of the Lung and GEP area. A series of at least 100 cases are available in our archives, and we shall expand the study to incoming cases. We shall perform in parallel in each case both, the novel RT-qPCR experiment and a battery of related IHC tests to be performed in highly reproducible procedures, using automatic apparatuses and a software for image analysis. The collected data will have great clinical impact, permitting the selection of appropriate surgical and chemo-therapeutic treatments and open prospects for the use of recently devised analogues of Somatostatin.

Objective

The present project is focused on a novel, fully original approach for the determination of data of diagnostic, prognostic and therapeutic interest in NET of the GEP area and lung. In place of a panel of immunohistochemical reactions, we intend to perform a Real Time quantitative (RT-q) PCR reaction on RNA extracted from FFPE tissue blocks. We shall order to commercial companies customized kits for the analysis of genes of our choice and the project is related to the use of kits whereby in a single experiment will be possible to detect and quantify the mRNA specific for a series of hormones, receptors, markers of proliferation and angiogenesis. Instead of performing a panel of immunohistochemical reactions, pathologists will be able to detect and quantify for each endocrine tumor the differentiation (specific hormone being produced, expression of receptors for somatostatin, GRP, potentialities for proliferation and angiogenesis).

Expected results

- Collection of minimum 100 cases of Neuro-endocrine tumors of the GEP area and lung. These cases will be collected from the Archives of the Babes Institute where an ample collection of Formalin-fixed Paraffin embedded blocks is duly preserved and classified. Cases of recent observation will also be provided from the Pathology Service of Central Military Hospital of Bucharest. These patients are going to be followed and treated by the Clinical staff of Elias Emergency University Hospital.
- The histological cases are going to be classified on a morphological basis by pathologists of the Babes Institute and of the Lotus Medical Centre. The study on a selected case series will then be conducted in parallel using immunohistochemical analysis (Lotus Medical Centre and Babes Institute) and a Molecular biological analysis (Babes Institute).
- The Immunohistochemical analysis will be conducted on tissue section using an Automatic Apparatus for immunostaining, permitting highly controlled and reproducible result. Tests will be performed for a series of differentiation markers (Chromogranin A, Synaptophysin, Insulin, Glucagon, Gastrin, PP, Somatostatin, NSE, PGP 9.5), of Receptors (SSTR 1,2,3,4,5) of Markers of origin (TTF1, INS1, CDX2) and of Proliferation (Ki67). The results will be analyzed using a software permitting image analysis and an objective evaluation of the data.
- On the same cases, RNA will be extracted form sections of the blocks and tested on customized arrays ordered to commercial Companies for the analysis of genes of our choice (see below) and using the Real-Time quantitative PCR procedure. The results will be read with a Stratagene Mx3005P Apparatus (already available at Babes Institute).
- Comparison of the Immunohistochemical vs. the RT-qPCR data and interpretation.
- Planning by the Clinicians of targeted interventions.

Result: patient application for a specific device dedicated to neuroendocrine tumors investigation.
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**Evaluarea prognosticului si a sensibilitatii la terapie in tumorile neuroendocrine.**
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