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OPTIMARK project: search for quality markers for paraffin-embedded tissue samples

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Background

Quality of tissue samples is a critical factor affecting the reproducibility of biomedical research results and the development of disease biomarkers. The multicentric OPTIMARK project, developed in the context of the Spanish Biobank Network, is focused on trying to find sensitive quality tissue markers, to evaluate pre-analytical factors, to gain a precise knowledge on the real quality of tissue samples, which will contribute to render not only robust scientific analytical results but an accurate biomarker development and analysis.
To this end, we are testing a number of biomarkers universally present in human tissues, by immunohistochemistry in paraffin-embedded tissues, trying to correlate the expression level with the time of storage of the samples from the time of collection to the final analytical use.
We already have established the criteria to select biomarkers to be tested for antigenicity analysis. In previous studies we analyzed ki67, Vimentin and CD31 in 374 non-tumoral tissues including lung, colon, stomach, endometrium, breast and brain. We only found a significant negative correlation between ki67 intensity and the time of storage in specific tissues (colon and stomach).

Materials and Methods

We selected 25 non-pathological cores of tissue from breast, lung and colon to try to find biomarkers associated to the time of storage, dividing the cases into 5 groups per tissue according to this pre-analytical variable (**Table1**). To facilitate, we generated 3 different Tissue Microarrays (TMA), including the 25 cases from each tissue type.

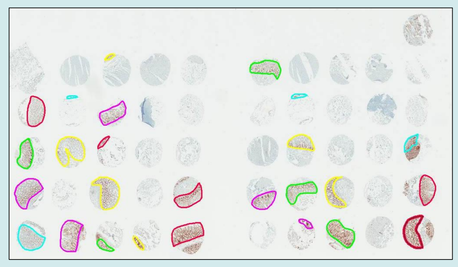
Table 1. *Stratification of the pre-analytical variable analysed.*

	CONTROL	GROUP A	GROUP B	GROUP C	GROUP D
Time of storage	< 1 year	1-5 years	5-10 years	10-20 years	>20 years

On these TMAs, we analysed the immunohistochemical (IHC) expression of different biomarkers related to structural proteins and cellular metabolism, present in as many tissues as possible according to literature, and, to allow automatic evaluation, we selected markers with a nuclear moderate-intense intensity of staining, to avoid signal saturation.

We finally selected DNA mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) (antibodies from Ventana Medical Systems, Roche. Benchmark®XT automatized staining) for testing.
We use the Aperio Digital Pathology Slide Scanner (Leica®) to scan the immunostained slides and the Aperio ImageScope Pathology Slide Viewing Software (Leica®) to select the areas where to perform the analysis (example of a TMA stained with selected areas in Figure 1). Automatic quantification of the IHC expression was performed using the analysis algorithms developed by Leica®. We used SPSS for statistical analysis, using ANOVA's test.

Figure 1. Selection of areas for analysis of a representative slide



Results

Figure 2. *RESULTS of mean of the percentage of intensively stained nuclei (3+) for the different biomarkers analyzed, as a function of the time of storage. Different colours indicate the tissue type.*

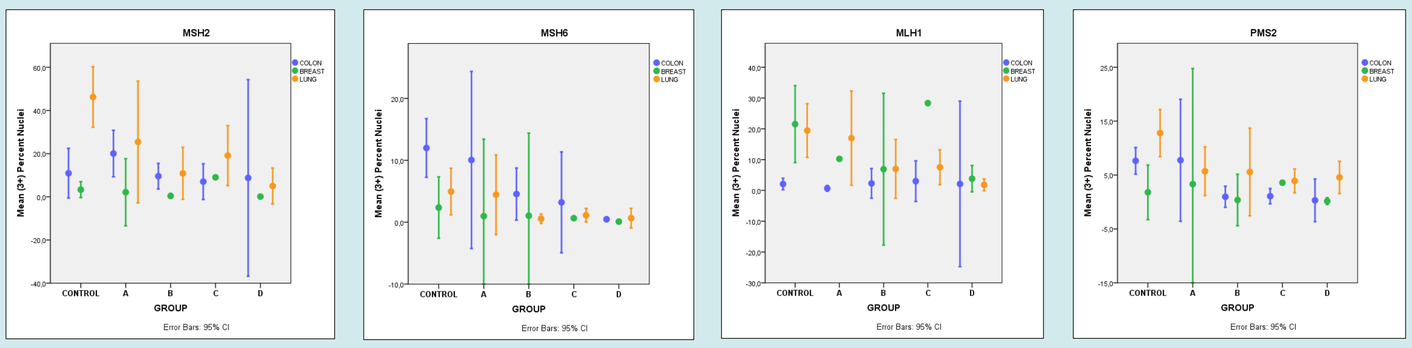
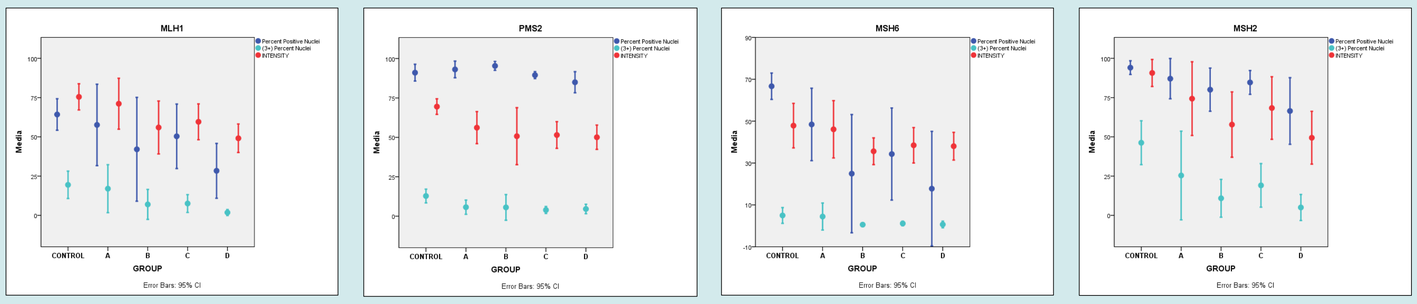


Figure 3. *RESULTS of expression in lung. IHC signal was evaluated as total percentage of stained nuclei, percentage of intensively stained nuclei (3+) and the mean of the intensity of the signal. ANOVA results are shown in tables.*



ANOVA[a]

		Suma de cuadrados	gl	Media cuadrática	F	Sig.
(3+) Percent Nuclei	Inter-grupos	1093,153	4	273,288	4,828	,007
	Intra-grupos	1132,205	20	56,610		
	Total	2225,358	24			
Percent Positive Nuclei	Inter-grupos	3902,765	4	975,691	2,899	,048
	Intra-grupos	6730,524	20	336,526		
	Total	10633,289	24			
INTENSITY	Inter-grupos	2361,594	4	590,399	5,493	,004
	Intra-grupos	2149,480	20	107,474		
	Total	4511,075	24			

a. Barcode ID = TMA PULMON-1 OPTIMARK:MLH1

ANOVA[a]

		Suma de cuadrados	gl	Media cuadrática	F	Sig.
Percent Positive Nuclei	Inter-grupos	2113,844	4	528,461	4,825	,008
	Intra-grupos	2285,449	20	114,272		
	Total	4399,293	24			
(3+) Percent Nuclei	Inter-grupos	5088,223	4	1272,056	7,008	,001
	Intra-grupos	3630,393	20	181,520		
	Total	8718,616	24			
INTENSITY	Inter-grupos	5016,346	4	1254,087	5,563	,004
	Intra-grupos	4508,871	20	225,444		
	Total	9525,217	24			

a. Barcode ID = TMA PULMON-1 OPTIMARK:MSH

ANOVA[a]

		Suma de cuadrados	gl	Media cuadrática	F	Sig.
Percent Positive Nuclei	Inter-grupos	7627,011	4	1906,753	6,207	,002
	Intra-grupos	6143,859	20	307,193		
	Total	13770,870	24			
(3+) Percent Nuclei	Inter-grupos	93,632	4	23,408	3,004	,043
	Intra-grupos	155,852	20	7,793		
	Total	249,484	24			
INTENSITY	Inter-grupos	585,475	4	146,369	2,468	,078
	Intra-grupos	1186,011	20	59,301		
	Total	1771,486	24			

a. Barcode ID = TMA PULMON-1 OPTIMARK:MSH

ANOVA[a]

		Suma de cuadrados	gl	Media cuadrática	F	Sig.
Percent Positive Nuclei	Inter-grupos	286,977	4	71,744	4,782	,008
	Intra-grupos	285,057	19	15,003		
	Total	572,035	23			
(3+) Percent Nuclei	Inter-grupos	256,347	4	64,087	5,613	,004
	Intra-grupos	216,934	19	11,418		
	Total	473,281	23			
INTENSITY	Inter-grupos	1300,957	4	325,239	5,936	,003
	Intra-grupos	1059,841	19	55,728		
	Total	2359,798	23			

a. Barcode ID = TMA PULMON-1 OPTIMARK:PMS2

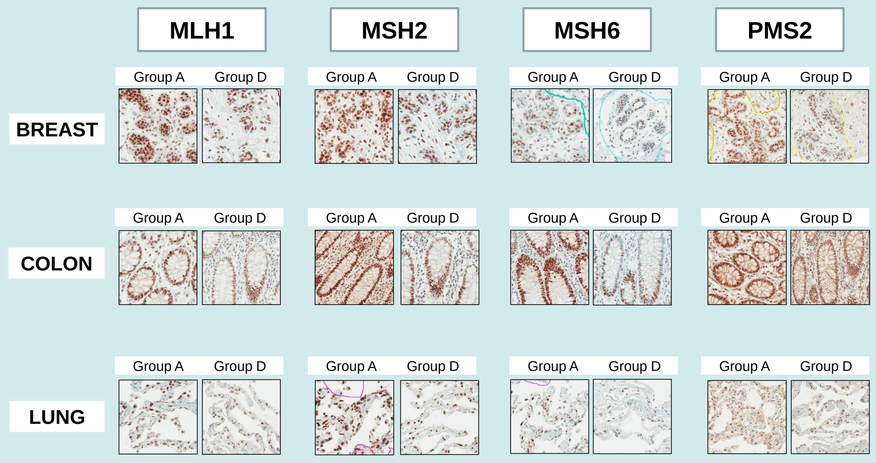


Figure 4. *Images showing the comparison of immunohistochemical stain between group A and D, for each marker, in breast, colon and normal lung tissue. A clear decrease in the expression of the long storage time tissue compared with control is found.*

MSH2 LUNG

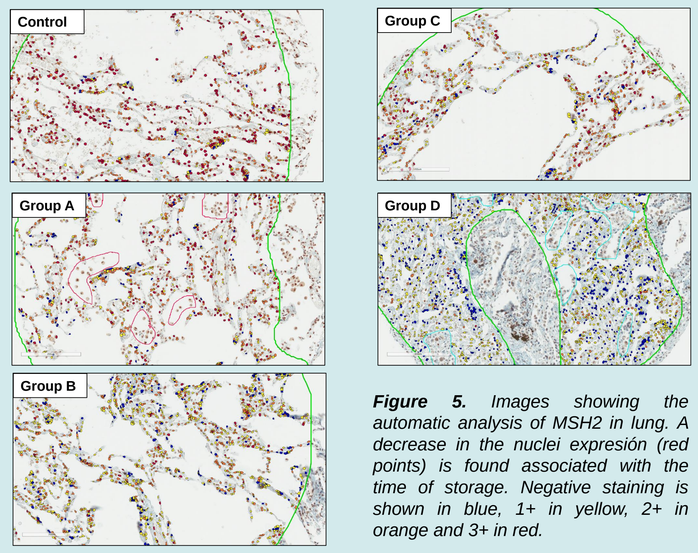


Figure 5. *Images showing the automatic analysis of MSH2 in lung. A decrease in the nuclei expresión (red points) is found associated with the time of storage. Negative staining is shown in blue, 1+ in yellow, 2+ in orange and 3+ in red.*

Conclusions

- We have been able to find some promising biomarkers that define the quality and antigenicity in storage paraffin embedded tissue. DNA mismatch repair proteins expression is negatively associated to the time of storage of paraffin-embedded non-pathological tissue samples, being statistically significant in lung tissues, although in colon and breast seems to be also sensitive to this preanalytical variable.
- Even that the staining for DNA mismatch repair protein allows the diagnosis, the intensity and the nuclei positivity decreases win longer storage tissue.
- Proteins related to cellular structure or central metabolism seem to be good candidates to be used as quality universal biomarkers. Besides, quantification of nuclear antigens expression could be easily automated, facilitating implementation in research and clinical practice.
- Ongoing work from our group is expanding this study to additional tissue types (brain and endometrium) and a a larger series of cases to confirm these results.