# Expression and Interaction of Claudin-3 and Occludin During the Colorectal Cancer Progression

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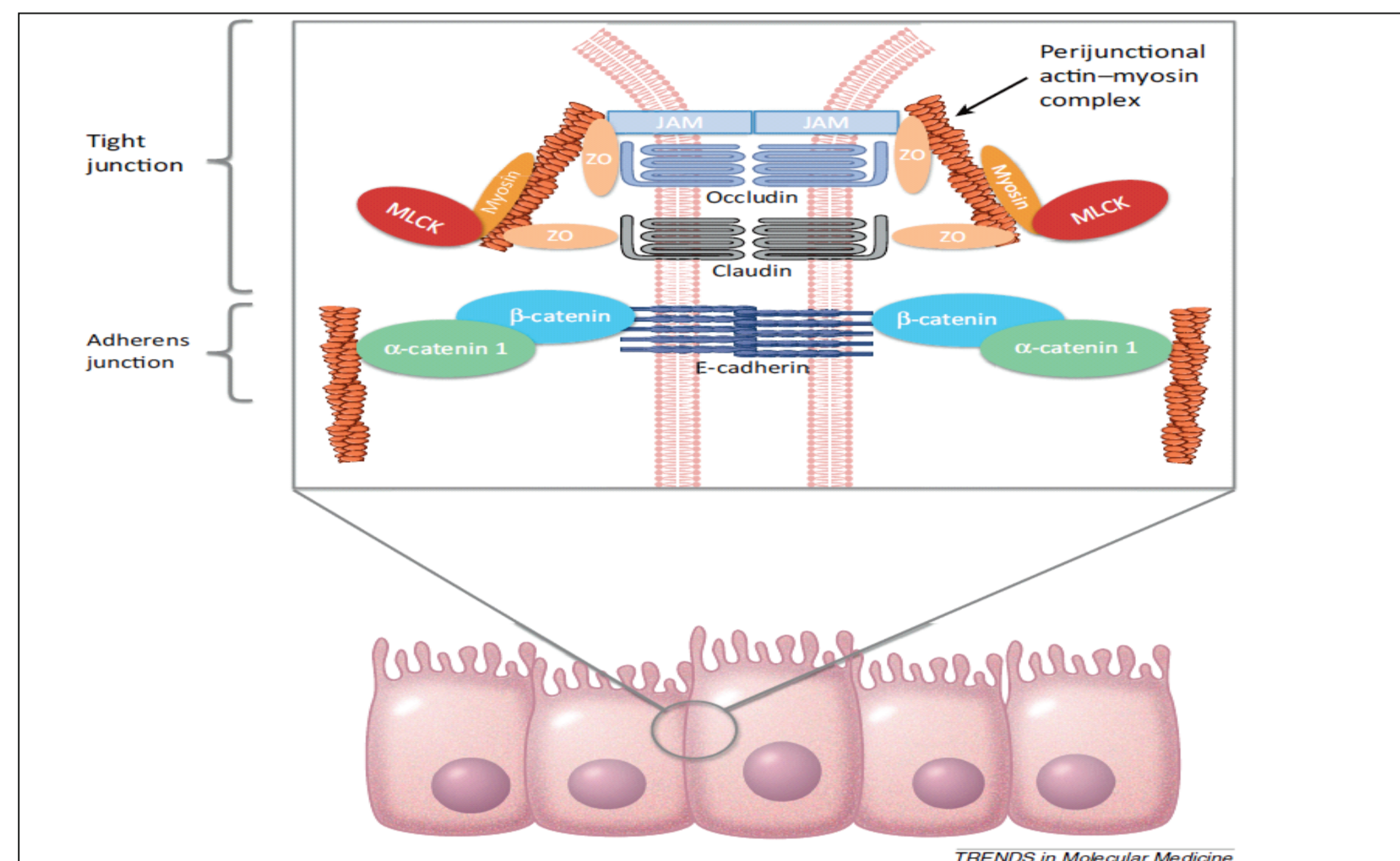
Figure 1: Molecular composition of Apical Junctional Complex. Schematic representation of the components of Apical Junctional complex, tight junction and adherens junction. Observe the main molecular constituents tight junction between the epithelial cells. Reference: <http://www.hindawi.com/journals/jo/2010/541957/fig1/>

Table 1: The clinicopathological features of colorectal cancer patients.

| Clinicopathological features	Numbers
Gender	
Male/Female	11/15
Age mean (years)	61
Location	
Ascending colon	12
Transverse colon	4
Descending colon	2
Sigmoid	5
Rectosigmoid	3
Histology	
Well	2
Moderately	18
Poorly	3
Mucinous	3
TNM stage	
0	1
I	2
II	10
III	13
IV	0
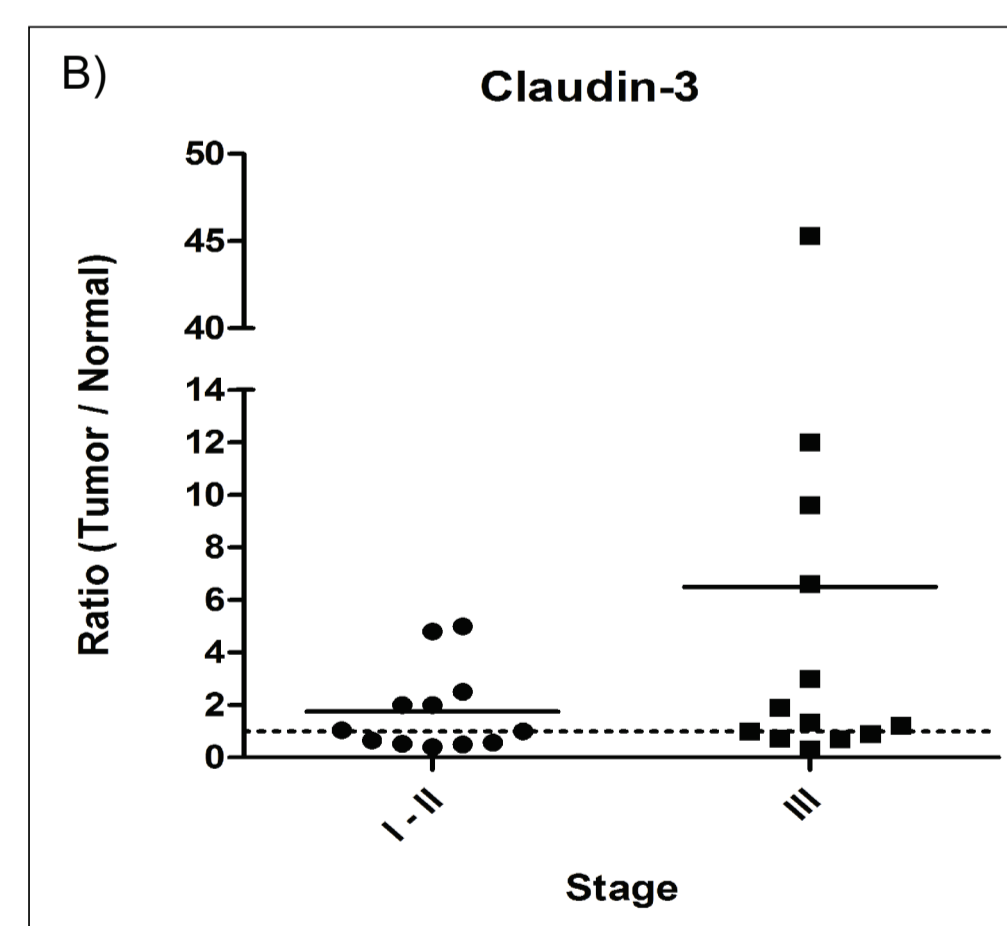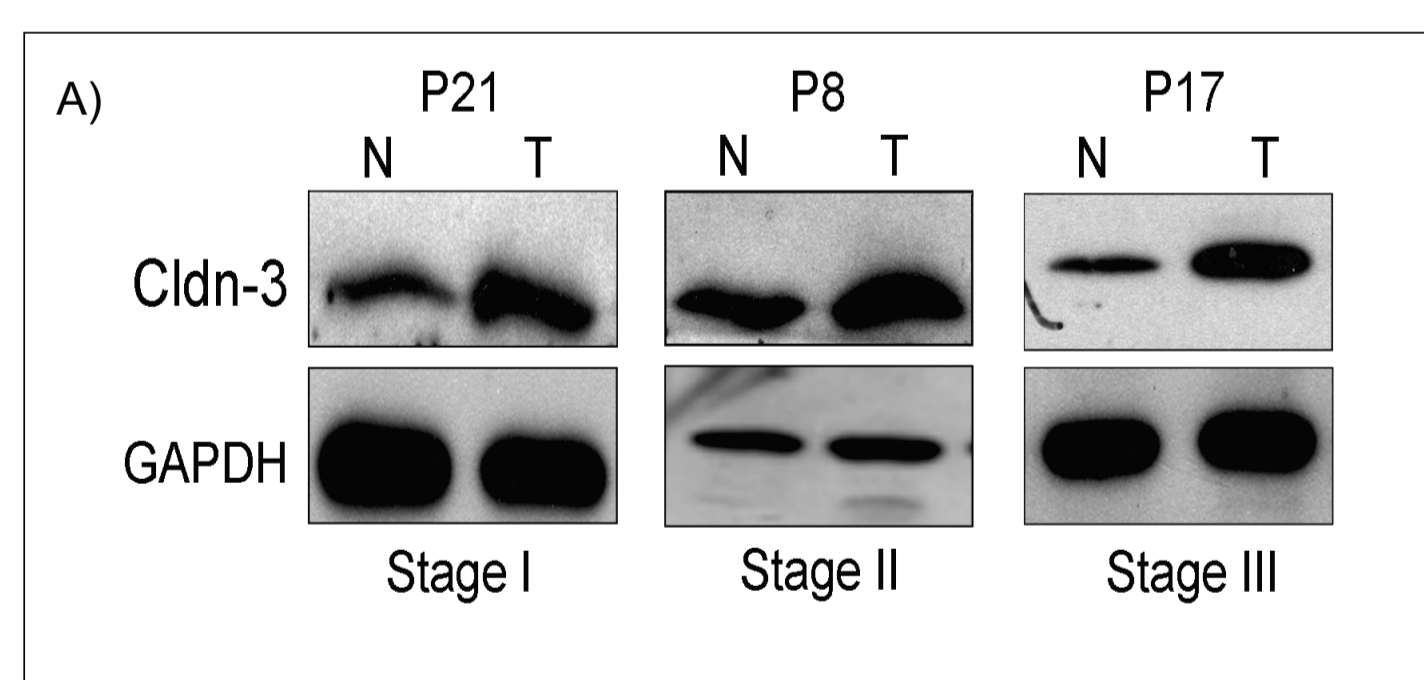
Figure 2: Correlation of the patients staging with Claudin-3 protein expression. a) Claudin-3 protein levels in normal and tumor tissues were analyzed by immunoblotting. Optical density of the Claudin-3 was normalized by GAPDH. b) Graphical representation of the TNM staging correlated with expression levels of protein component of tight junctions. The values plotted on the graph correspond to ratio of expression in the tumor to normal tissue. The dotted line correspond to value = 1. The mean for each group is represented on the graph. Number of patients: stage I - II (n=12), stage III (n=13).
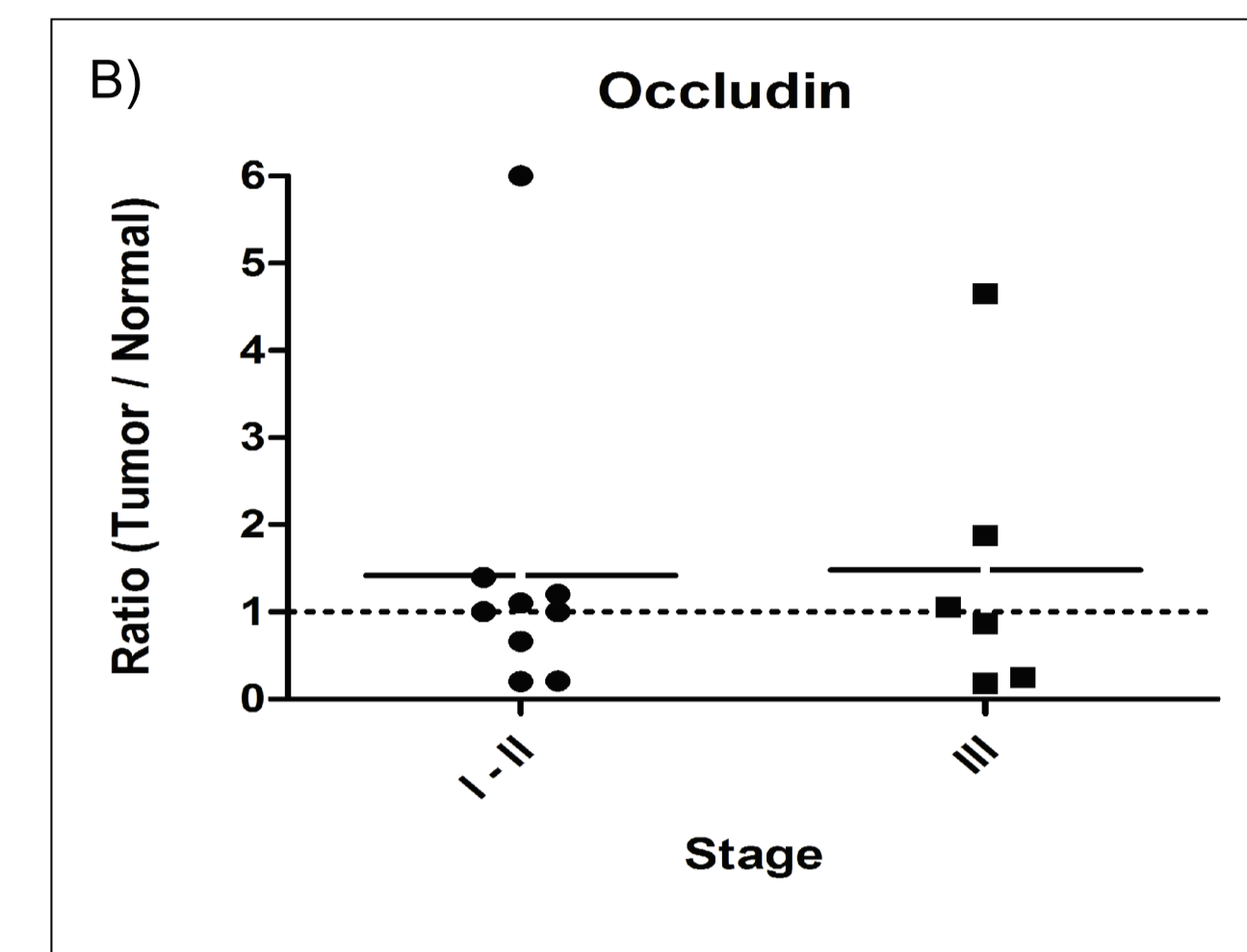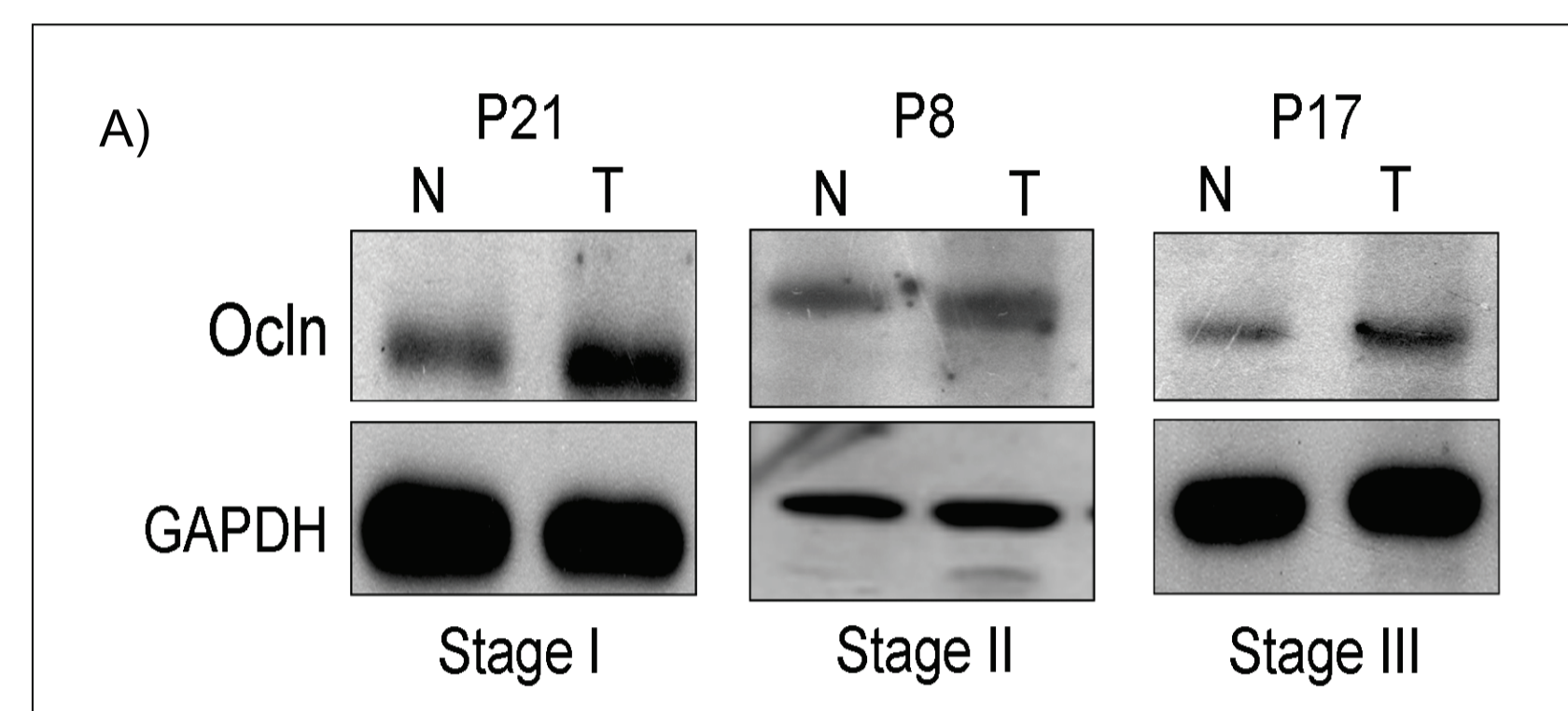

Figure 3: Correlation of the patients staging with Occludin protein expression. a) Occludin protein levels in normal and tumor tissues were analyzed by immunoblotting. Optical density of the occludin was normalized by GAPDH. b) Graphical representation of the TNM staging correlated with expression levels of protein component of tight junctions. The values plotted on the graph correspond to ratio of expression in the tumor to normal tissue. The dotted line correspond to value = 1. The mean for each group is represented on the graph. Number of patients: stage I - II (n=9), stage III (n=6).
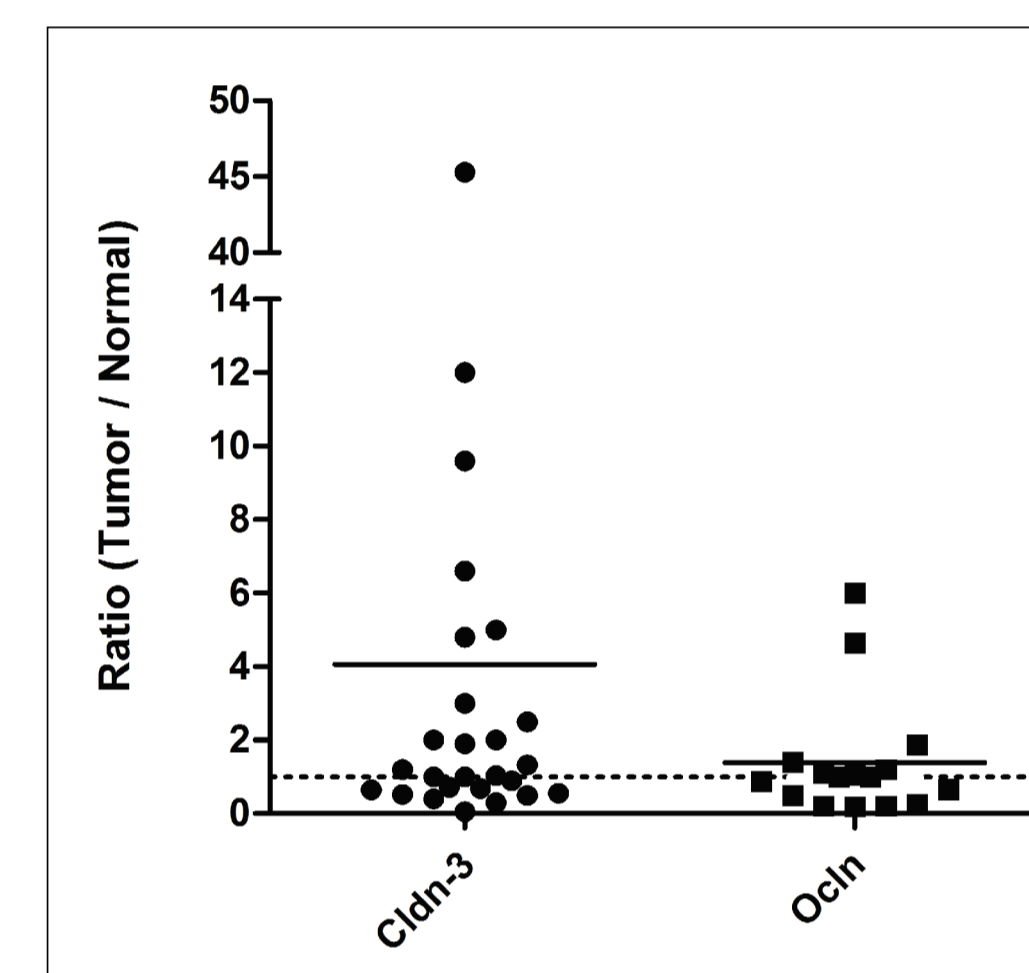
Figure 4: Analysis of the claudin-3 and occludin proteins levels of patient samples used for Western blotting. Optical densities of the occludin and claudin-3 were normalized by GAPDH. The values plotted on the graph correspond to ratio of expression in the tumor to normal tissue. The dotted line correspond to value = 1. The mean for each group is represented on the graph. Number of patients: claudin-3 (n=26), occludin (n=16)
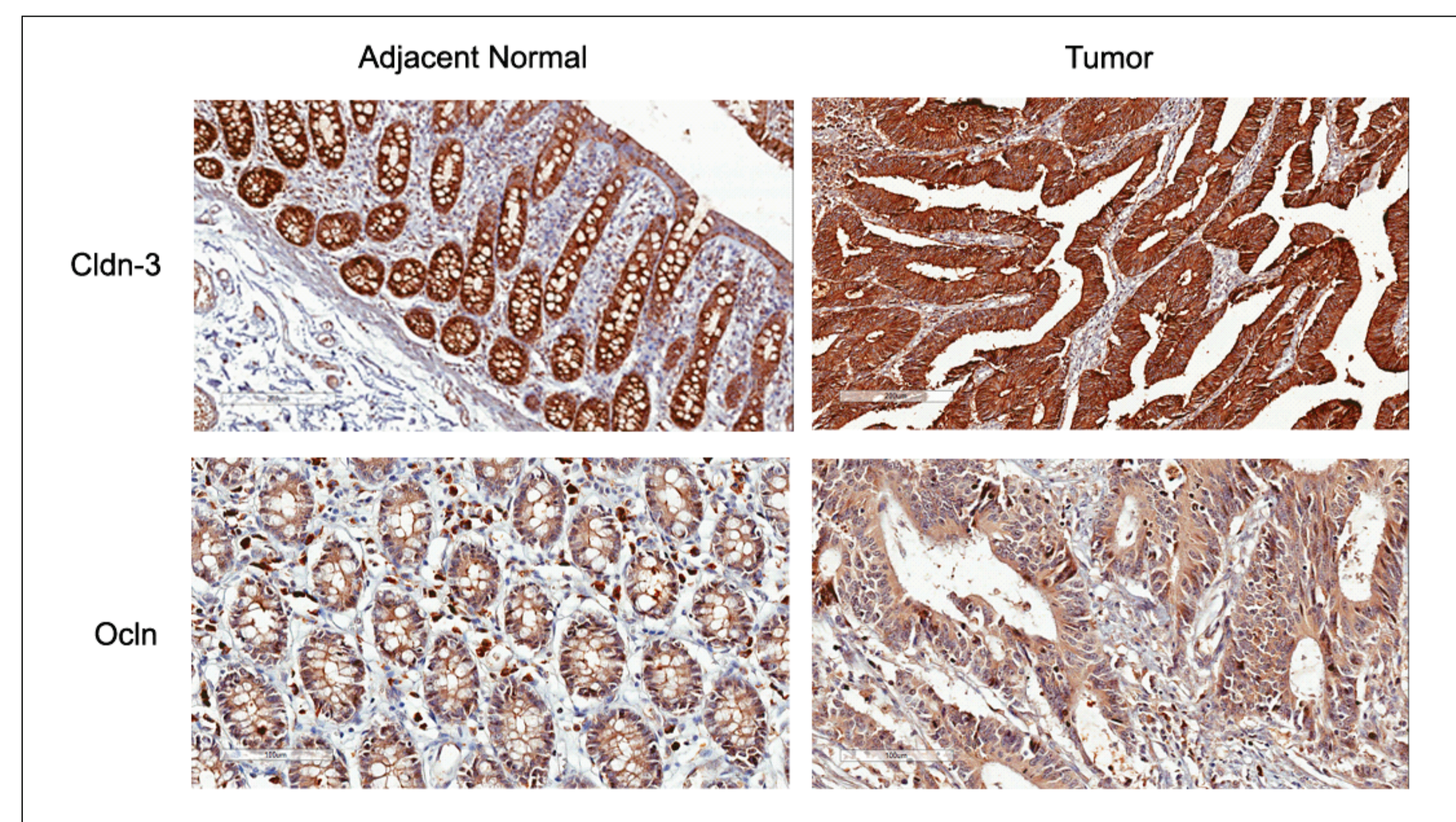
Figure 5: Expression of Claudin-3 and Occludin in patient tissues. The expression of proteins was analyzed by IHC in the colorectal cancer tissues and paired non-colorectal cancer tissue. The staining for claudin-3 and occludin proteins was performed in the same patient.
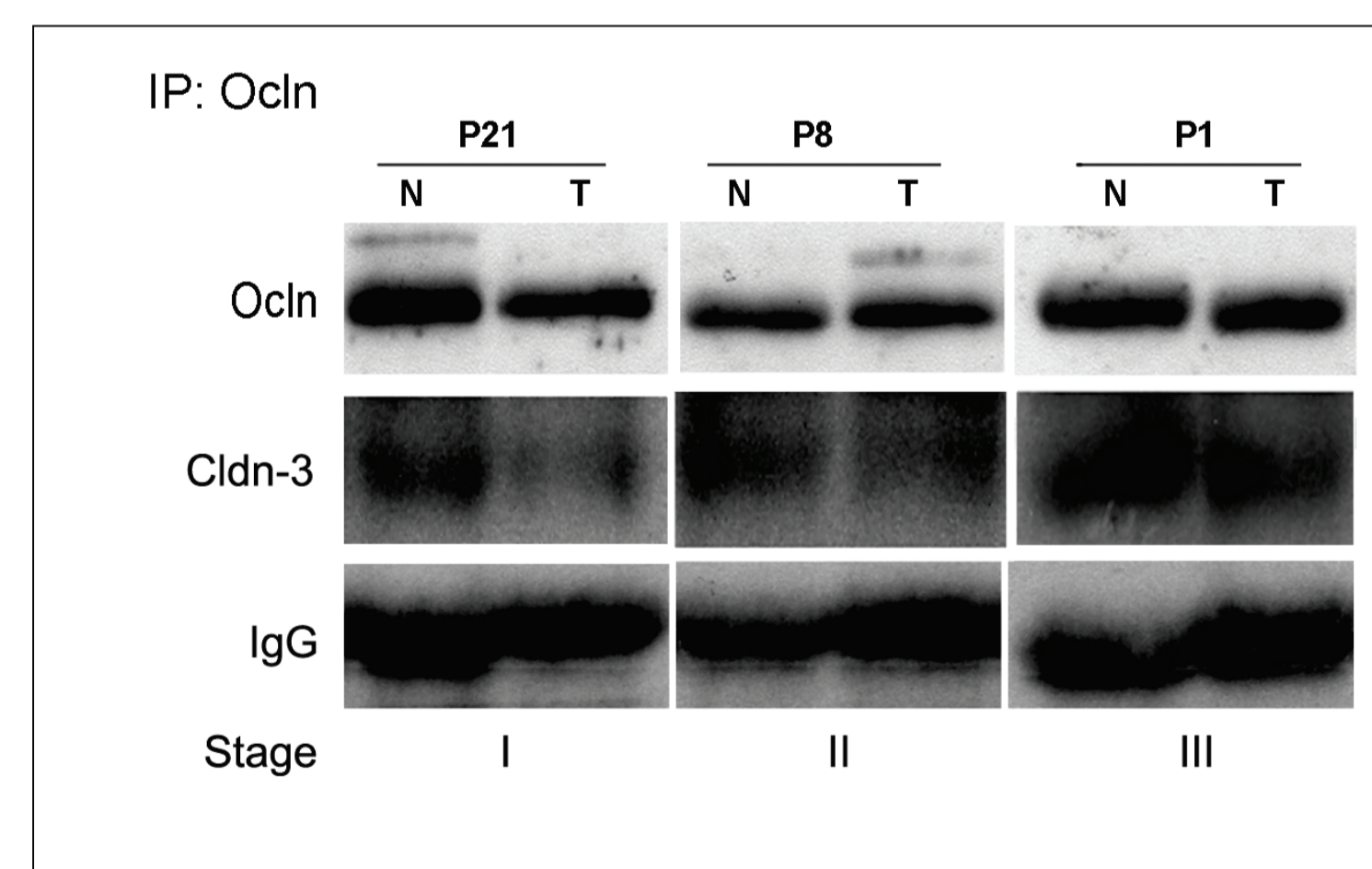
Figure 6: The interaction between tight junction proteins. The total poteins lysates of the patient samples was performed for immunoprecipitation using an antibody anti-occludin. The analysis of the claudin-3 and occludin protein levels in the patient samples were made by immunobloting.

BACKGROUND: During colorectal cancer (CRC) progression, epithelial cells undergo cell-cell adhesion disassembly increasing their malignant potential. In this context, the claudins and occludin (tight junctions proteins) play important role regulating events related with carcinoma progression. Previous studies have showed altered expression of claudin proteins in human CRC samples. Nevertheless, the molecular interactions that modulating the Tight Junction (TJ) functions and their role regulating the malignant potential remain to be defined.

AIMS: Evaluate the importance of expression and interaction of the claudin-3 and occludin proteins during the colorectal cancer progression.

METHODS: Human colorectal specimens were obtained from surgical resection of CCR patients treated in Brazilian National Cancer Institute (INCA in portuguese). In all cases, we collected adenocarcinoma specimens and their paired normal mucosa, which were classified by TNM staging. Claudin-3 and occludin protein levels were analyzed by imunoblotting and the interaction between these proteins were evaluated by immunoprecipitation. This study is being carried out with approval of the INCA Research Ethics Committee (Prot 84/04).

RESULTS: Our results showed that samples in the earliest stage presented decreasing of claudin-3 and occludin expression in tumors. Nevertheless, samples in advanced stage presented a raised expression of these proteins in tumors. Moreover, we observed decreasing of interaction between claudin-3 and occludin in tumors during all stages of this disease.

CONCLUSION: Together, our findings indicate that during CRC progression there is increase of claudin-3 and occludin expression in tumor tissue, which is accompanied by decrease of interaction between these proteins.