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pRb2/p130 Localizes to the Cytoplasm in Diffuse Gastric Cancer

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pRb2/p130 is a key tumor suppressor, whose oncosuppressive activity has mainly been attributed to its ability to negatively regulate cell cycle by interacting with the E2F4 and E2F5 transcription factors. Indeed, pRb2/p130 has been found altered in various cancer types in which it functions as a valuable prognostic marker. Here, we analyzed pRb2/p130 expression in gastric cancer tissue samples of diffuse histotype, in comparison with their normal counterparts. We found a cytoplasmic localization of pRb2/p130 in cancer tissue samples, whereas, in normal counterparts, we observed the expected nuclear localization. pRb2/p130 cytoplasmic delocalization can lead to cell cycle deregulation, but considering the emerging involvement of pRb2/p130 in other key cellular processes, it could contribute to gastric tumorigenesis also through other mechanisms. Our data support the necessity of further investigations to verify the possibility of using pRb2/p130 as a biomarker or potential therapeutic target for diffuse gastric cancer.
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Gastric cancer is still one of the most frequent causes of cancer death among women and men (Pinheiro et al., 2014). The most frequently used gastric cancer classification, which is based on Lauren's criteria, distinguishes two main groups that differ not only in morphology but also clinically and epidemiologically: the intestinal and the diffuse histotypes (Lauren, 1965; Vauhkonen et al., 2006). The first has a glandular morphology and is characterized by a stepwise progression, whereas gastric tumors of the diffuse histotype are characterized by an undifferentiated morphology, the lack of precursor lesions, occur most commonly in young patients and generally have a worse prognosis (Nardone, 2003; Vauhkonen et al., 2006; Chiaravalli et al., 2012; Corso et al., 2012). In the clinical practice however, regardless of histotype, the clinical stage seems to be the most important single and independent factor affecting survival (Vauhkonen et al., 2006). Although various markers for early diagnosis have been discovered (Guilford et al., 1998; Shafaghi et al., 2013; Liu et al., 2014), the lack of remarkable early symptoms still results in a late stage diagnosis. Therefore, it is a priority to identify new tools for an early diagnosis.

pRb2/p130 is a member of the Rb family of tumor suppressors (Giordano et al., 1991; Yeung et al., 1993; Indovina et al., 2013), whose altered expression and delocalization was found in various cancers. In particular, reduced expression of

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pRb2/p130 has been found in breast and endometrial (Susini et al., 1998; Milde-Langosch et al., 2001; Susini et al., 2001), brain (Li et al., 2004), ovarian (D'Andrilli et al., 2004), lung (Claudio et al., 2000; Caputi et al., 2002), prostate (Claudio et al., 2002), salivary gland cancers (Russo et al., 2005), vulvar squamous cell cancer (Zamparelli et al., 2001), choroidal (Massaro-Giordano et al., 1999) and oral mucosa melanoma (Tanaka et al., 2001), and soft tissue sarcomas (Masciullo et al., 2008). In various instances, reduced expression of pRb2/p130 was shown to inversely correlate to tumor grading and to be a prognostic factor indicating a poor overall survival. Interestingly, a cytoplasmic localization, entailing a loss of function, was found in various tumor types including lymphoma cell lines (Cinti et al., 2000). Consistently, pRb2/p130 is normally localized in the nucleus (Baldi et al., 1995, 1996) and its oncosuppressive activity has mainly been correlated with its ability to negatively regulate the cell cycle through the interaction with the E2F4 and E2F5 transcription factors (Dyson, 1998). This function of pRb2/p130 is regulated through changes in its phosphorylation status: when hypophosphorylated, pRb2/p130 binds E2F4 and E2F5 transcription factors, releasing them after phosphorylation (Giacinti and Giordano, 2006; Sun et al., 2007). Thus, pRb2/p130 loss of function, independently from causes, could determine an uncontrolled transcription of E2F4 and E2F5 target genes, which include genes involved in cell cycle progression (Sardet et al., 1995; Claudio et al., 1996; Takahashi et al., 2000). Moreover, beyond its function as cell cycle regulator, pRb2/p130 is also involved in many other cellular processes, such as regulation of apoptosis, senescence, and differentiation, which could all contribute to the pRb2/p130 oncosuppressive activity (Indovina et al., 2013).

In 2007, we analyzed the expression of various cell cycle regulated genes, including pRb2/p130, in gastric cancer and found that in tumors of the intestinal type, pRb2/p130 cytoplasmic localization correlated with the expression levels of EZH2, a member of the Polycomb group family of transcriptional regulators (Matsukawa et al., 2006; Guo et al., 2014), and VEGF, which both have a recognized role in gastric carcinogenesis (Chen et al., 2014; Mao et al., 2014). Here, we focused specifically on the analysis of pRb2/p130 expression in a wider series of gastric tumors of the diffuse histotype.

Materials and Methods

Tissue sample collection

Thirty eight tissue samples from patients (15 women and 23 men) undergone to surgery in 2004 at the Monaldi hospital were collected. All the patients suffered from gastric cancer of diffuse histotype. TNM classification parameters are the following: nine samples were classified as T1 (all of them were N0), 18 as T2 (9 N0, 1 N1, 7 N2, and 1 N3), seven as T3 (3 N1, 3 N2-one of which was M1- and 1 N3), and five as T4 (3 N1 and 2 N2).

Immunohistochemistry

Sections of each sample, cut from the same blocks, were used to perform immuhistochemical reactions, according to the protocol previously described in Mattioli et al. (2007). The antibody anti-pRb2/p130 (Abcam, code ab17124, mouse monoclonal) was used at a 1:25 dilution and incubated 1 h at room temperature.

Statistical analysis

After checking data normality by the D'Agostino-Pearson normality test, statistically significant differences were evaluated by the one-way repeated measures Anova with Tukey post-test (GraphPad Prism Software, version 5.01 for Windows), which is appropriate to compare the means of multiple matched groups; $P < 0.05$ was considered to be statistically significant.

Results

pRb2/p130 accumulates in the cytoplasm of gastric cancer cells

We analyzed pRb2/p130 expression in 38 gastric cancer samples and their adjacent normal tissues by immunohistochemical analysis. Results showed a marked cytoplasmic localization of pRb2/p130 in cancer tissues (Fig. 1), whereas normal adjacent tissues showed the expected nuclear localization (Baldi et al., 1995, 1996). In particular, in non-tumor samples the mean percentage of cells with pRb2/p130 nuclear staining was significantly higher than the mean percentage of cells with cytoplasmic staining ($73.95\% \pm 5.71\%$ vs $26.05\% \pm 5.71\%$, $P < 0.001$) (Fig. 2). Conversely, in tumors the mean percentage of cells with pRb2/p130 nuclear expression was significantly lower than that with cytoplasmic expression ($25.00\% \pm 8.05\%$ vs $76.05\% \pm 8.63\%$, $P < 0.001$) (Fig. 2). Consistently, the mean percentage of cells with nuclear pRb2/p130 was significantly higher in normal cells than in cancer cells ($73.95\% \pm 5.71\%$ vs $25.00\% \pm 8.05\%$, $P < 0.001$) and the mean percentage of cells with cytoplasmic pRb2/p130 was significantly lower in normal cells than in cancer cells ($26.05\% \pm 5.71\%$ vs $76.05\% \pm 8.63\%$, $P < 0.001$).

A relationship between the nuclear and cytoplasmic levels of pRb2/p130 and tumor size and lymph node involvement was also investigated but no significant correlation was detected (data not shown).

Our results suggest that pRb2/p130 cytoplasmic localization could be a possible biomarker of stomach cell transformation, because of the sharp difference in pRb2/p130 localization between normal and tumor tissue samples.

Discussion

pRb2/p130 deregulation underlies different neoplasms and it is now well-established that it represents a crucial factor in the pathogenesis of multiple cancer types for which it could function as a diagnostic, prognostic or predictive factor. Although pRb2/p130 oncosuppressive role has been mainly attributed to its activity as cell cycle regulator (Paggi and Giordano, 2001; Cito et al., 2010), it is becoming increasingly clear that pRb2/p130 could contribute to tumor suppression by other mechanisms including apoptosis, senescence, and differentiation (Indovina et al., 2013), which all represent important antitumoral barriers.

Here, we focused on investigating the expression of pRb2/p130 in diffuse gastric cancer tissue samples, compared with adjacent normal tissues. The obtained results clearly showed a significantly prevailing cytoplasmic localization of pRb2/p130 in neoplastic areas, whereas normal tissue areas showed the expected prevailing nuclear localization. This is consistent to what found in other tumors. However, while in salivary gland tumors, for example, cytoplasmic expression of pRb2/p130 correlated with tumor grading, with the presence of metastasis and a with decreased probability of survival (Russo et al., 2005), here we did not find a correlation between tumor staging and higher percentage of cytoplasm localization. Also, unfortunately, survival data were not available for our patient group.

Overall our analysis shows a striking difference between pRb2/p130 localization in normal and tumor tissues, suggesting that pRb2/p130 has an important role in gastric tumorigenesis although its contribution in the development/progression of gastric cancer needs to be further elucidated and pRb2/p130 subcellular delocalization could not only

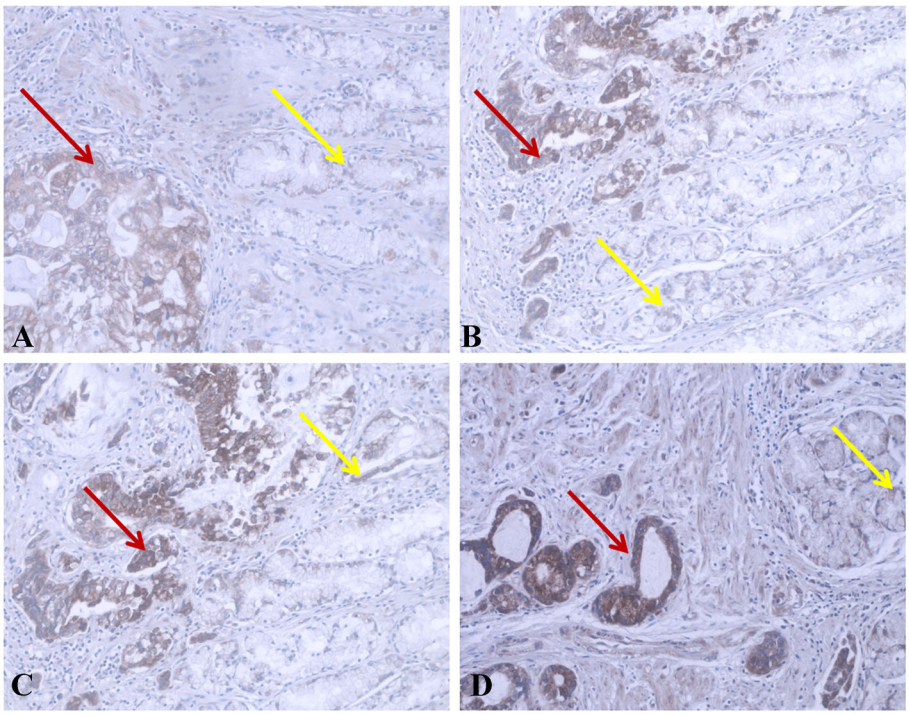

Fig. 1. Representative micrographs of immunohistochemical analyses of pRb2/p130 in diffuse gastric cancer. (A–D) Parts show a strong cytoplasmic localization of pRb2/p130 in diffuse gastric cancer areas (red arrows) compared with its nuclear localization in adjacent normal tissue areas (yellow arrows).

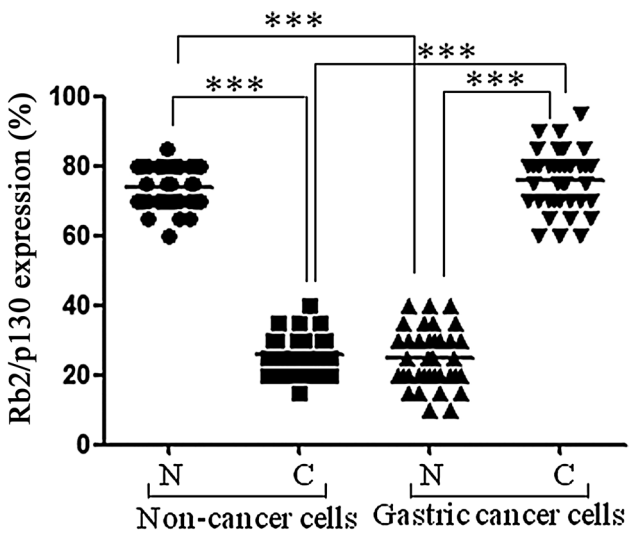

Fig. 2. Statistical evaluation of nuclear (N) and cytoplasmic (C) expression of pRb2/p130 in gastric cancer samples and their non-cancerous adjacent tissues. The dot plots report the percentage of cells expressing pRb2/p130 both individually for each sample (symbols) and as mean values for each group (lines). Statistically significant differences were evaluated by one-way repeated measures Anova with Tukey post-test and are indicated with *: extremely significant ($P < 0.001$).**

affect cell cycle regulation but also many other cellular processes.

In conclusion, we believe that pRb2/p130 cytoplasmic localization in diffuse histotype gastric cancer deserves further investigations because it might represent not only a potential marker for this kind of neoplasm but also a therapeutic target.

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