Western blotting

Total cellular extracts were prepared by directly lysing cells from dishes in SDS lysis buffer. Alternatively cells were lysed in either E1A buffer (25 mM HEPES pH 7.0, 0.1% NP-40, 150 mM NaCl, plus protease inhibitor cocktail; Calbiochem) or RIPA buffer (50 mM Tris HCl pH 7.4, 1% NP-40, 150 mM NaCl, 1 mM EDTA, plus protease inhibitor cocktail; Calbiochem). For western blotting, 0.45 μm nitrocellulose membrane (Schleicher and Schuell) was used and membranes were blocked for 1 hour at 37°C in 10% milk/PBS followed by incubation with the appropriate primary antibody diluted in 10% milk/0.5% Tween 20 for 1 hour. After several washings with PBS 0.5% Tween 20, HRP-conjugated secondary antibodies (DAKO) in 10% milk/0.5% Tween 20 were incubated for 1 hour. Blots were developed using Amersham ECL reagents according to the manufacturer's instructions.

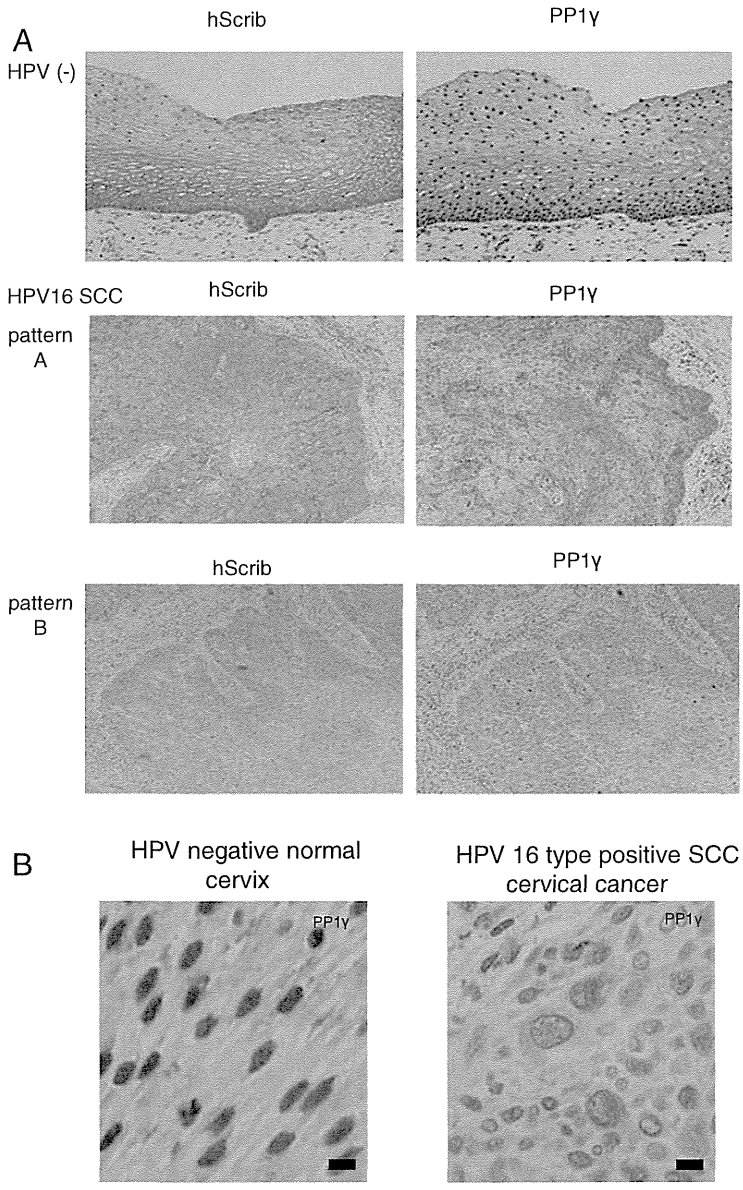

Figure 1 Immunohistochemical analysis of the expression and localisation of hScrib and PP1γ in advanced squamous cervical carcinomas. **(A)** Paraffin embedded excised tissues were immunostained with anti-hScrib or anti-PP1γ as indicated, and counterstained with haematoxylin. For the antibodies, immunostaining was performed according to standard techniques using an autostainer (BenchMark XT; Ventana Medical Systems, Inc., Tucson, AZ, USA). Representative experiments for a section of cervical epitheliums from normal cervix and advanced squamous cervical carcinomas (×200 original magnification). **(B)** High resolution microscopic images (scale bars: 20 μm) for a section of cervical epitheliums from normal cervix and HPV-16 positive advanced squamous cervical carcinomas.

Immunohistochemistry

All tissue samples were fixed in formalin and embedded in paraffin (obtained from patients under Institutional Review Board approval through the University of Tokyo Hospital). For all antibodies, immunostaining was performed according to standard techniques using an autostainer (BenchMark XT; Ventana Medical Systems, Inc., Tucson, AZ, USA). Immunoreactivity was interpreted based on the negative control, which was incubated without the primary antibody. Detection of hScrib expression was evaluated based on the existence of basolateral membrane staining as described previously [28]. For PP1γ, the expression was evaluated by nuclear staining. The immunostaining patterns of each sample were evaluated independently and blindly by pathologists specializing in gynaecological pathology, and cytology.

PCR-based HPV DNA testing

DNA was extracted from cervical smear samples by using the QIAGEN® DNeasy® Blood & Tissue Kits. PCR-based HPV DNA testing was performed using the PGMY-CHUV assay. Briefly, standard PCR was conducted using the PGMY09/11 L1 consensus primer sets and HLA-dQ primer sets. Reverse blotting hybridization was subsequently performed as described previously [33].

Results

Distribution patterns of hScrib and PP1γ in HPV-16 positive cervical intraepithelial neoplasm (CIN) and cervical carcinoma tissues

Previous studies had highlighted hScrib as a potential biomarker for HPV-16 induced malignancy [19,28,34]. We reasoned that if PP1γ was also regulated directly by hScrib, this should be similarly affected in HPV-16 induced malignancy. In order to investigate this we performed IHC analysis of hScrib and PP1γ expression in HPV-16 positive cervical tumours and control cervix. The results obtained are shown in Figure 1 and Table 1. In normal tissue hScrib is found primarily at cell-cell junctions, with high levels of expression as the cells begin to differentiate. However, hScrib distribution is altered significantly in all the HPV-16 positive tumours, with significant redistribution in the pattern of expression in 5/11 tumours and a complete loss of expression in 6/11 tumours. These results are largely in agreement with previous studies [28,35]. In the case of PP1γ, this displays a largely nuclear pattern of expression and this is present throughout the differentiating epithelium in the normal cervical tissue. In contrast, in the cervical tumours there is a complete loss of expression of PP1γ in 2/11 cases, with a striking redistribution in the pattern of expression in the remaining 9 samples, where there was a shift from a nuclear localisation to a cytoplasmic pattern of expression.

Table 1 Immunostaining patterns for hScrib and PP1γ in clinical samples of human uterine cervix

hScrib		
	Normal	**16-positive**
Membrane	8	0
Cytoplasm	0	5
Nuclear	0	0
No expression	0	6
Total	8	11
PP1γ		
	HPV negative	**16-positive**
Membrane	0	0
Cytoplasm	0	9
Nuclear	8	0
No expression	0	2
Total	8	11

We were then interested in investigating whether perturbation in the pattern of PP1γ expression was an early or late event during HPV-induced neoplastic progression. To do this we repeated the PP1γ IHC analysis on lesions exhibiting different grades of CIN. The lesions were classified as CIN1 (n = 4), CIN2 (n = 8), CIN3 (n = 8). As shown in Figure 2, there is a marked loss in nuclear PP1γ expression, which is already apparent in CIN2, and this is more evident in the CIN3 lesion, where there are also much lower levels of PP1γ expression. Interestingly, PP1γ positive cells were distributed only in the lower third of the epithelial layer in CIN1 cases (4/4) and 8/8 of patients with CIN3 had PP1γ positive cells distributed in the lower, middle, and upper third of the epithelium (Figure 2B). In the case of hScrib, there is a similar perturbation in the pattern of expression as the lesions develop, but similar to what has been reported previously, there is a tendency in some lower grade lesions to find highly overexpressed hScrib in regions of the epithelium.

These results indicate that hScrib and PP1γ, whilst both being perturbed during the progression to malignancy, are altered in a manner that is not interdependent, suggesting that PP1γ might be an independent marker for cervical tumour development. Indeed, the pattern and expression levels of PP1γ declined with an almost linear relationship from normal tissue, through increasing grades of CIN lesion, to invasive cancer.

Analysis of PP1γ expression in HPV-16-positive cells

In order to determine whether perturbation of PP1γ expression was a direct result of HPV-16 oncoprotein function, we proceeded to examine the pattern of PP1γ expression in cell lines derived from HPV-16 positive cervical tumours. To do this we analysed the pattern of PP1γ expression in HPV-16 positive CaSki and SiHa

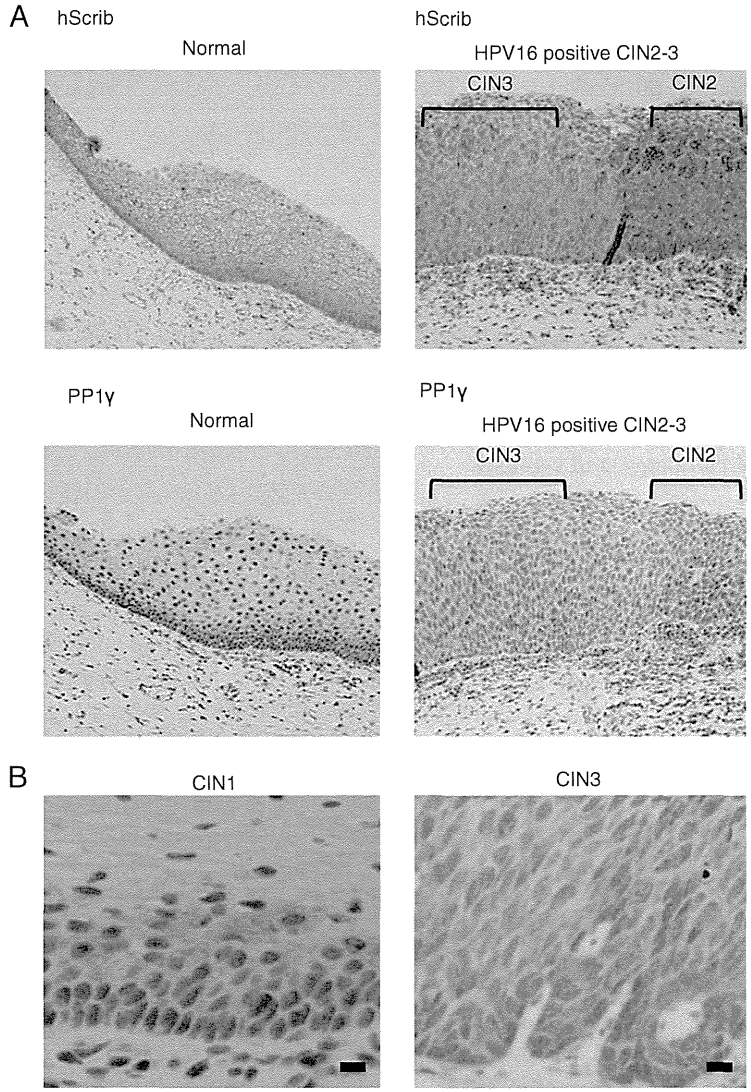

Figure 2 Immunohistochemical analysis of the expression and localisation of hScrib and PP1γ in various stages of cervical intraepithelial neoplasms. (A) Paraffin embedded excised tissues were immunostained with anti-hScrib or anti-PP1γ as indicated, and counterstained with haematoxylin. For the antibodies, immunostaining was performed according to standard techniques using an autostainer (BenchMark XT; Ventana Medical Systems, Inc., Tucson, AZ, USA). Representative experiments for a section of cervical epitheliums from normal cervix (left) and cervical intraepithelial neoplasms (CIN) grade 2 and 3 (right) (×200 original magnification). **(B)** High resolution microscopic images (scale bars: 20 μm) for a section of cervical epithelia from CIN grade 1 and 3.

cells, and compared this with HPV negative HaCaT cells. To determine whether any alterations might be HPV-specific, we also transfected the cells with siRNA E6/E7 and siLuc as a control. After 72 hours the cells were harvested and cells fractionated into cytosolic, membrane, nuclear and cytoskeletal pools, such that the pattern of PP1γ subcellular distribution could be monitored. The pattern of PP1γ expression was then ascertained by western blotting and the results obtained are shown in Figure 3. PP1γ is found predominantly within the nucleus in HaCaT cells (Figure 3A), whilst in the HPV-16 positive cells it is found weakly re-localised both in nuclear and cytoplasmic locations. However when E6/E7 expression is ablated there is a dramatic redistribution in the pattern of PP1γ expression, with much higher levels being found within the nuclear fraction of the cells (Figure 3B). In contrast, we found no difference in PP1γ transcript levels after siRNA E6/E7 treatment in HPV-16 positive cells (data not shown).

These results suggest that loss of nuclear PP1γ expression in HPV positive tumour cells is a direct result of the expression of the HPV E6/E7 oncoproteins.

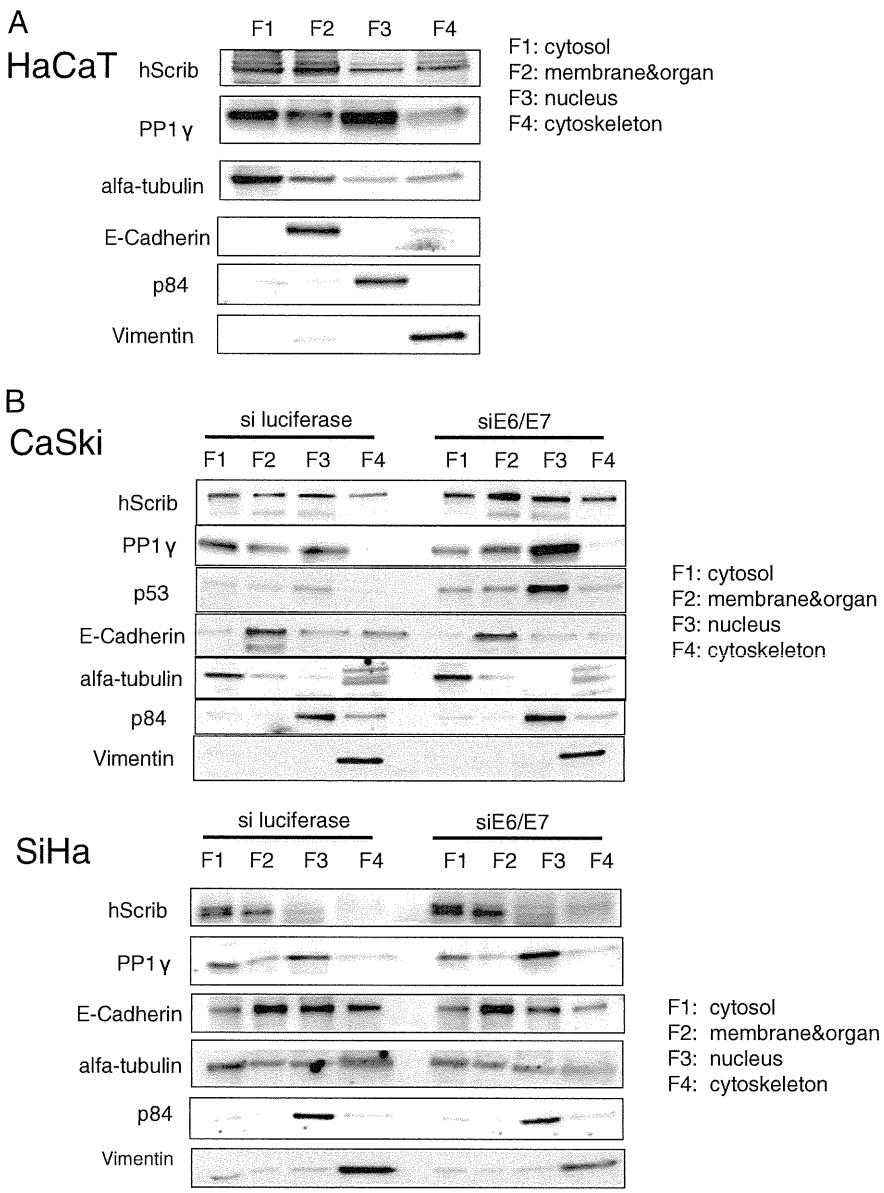

Figure 3 PP1γ is mislocalised in HPV-16 positive tumour cells. (A) HaCaT cells, and **(B)** siE6/E7or siluciferase control transfected CaSki and SiHa cells were fractioned into cytoplasmic (F1), membrane (F2), nuclear (F3), and cytoskeleton (F4) pools and hScrib and PP1γ were detected by western blotting. α-tubulin was a loading control for the cytoplasmic fraction, E-Cadherin was a loading control for the membrane fraction, p84 was a loading control for the nuclear fraction, and Vimentin was a loading control for the cytoskeleton fraction.

PP1γ is subject to degradation in HPV-16 positive cells

Interestingly, the fractionation studies indicate that whilst there is a significant increase in nuclear PP1γ in the absence of E6/E7, there is not a significant loss of cytoplasmic PP1γ, suggesting that some of the loss of nuclear expression may be due to proteasome mediated degradation. Therefore we were first interested in determining whether E6/E7 expression could affect the total levels of PP1γ expression. To do this we analysed the levels of PP1γ expression in total cell extracts from CaSki and SiHa cells previously transfected with siRNA E6/E7 or siLuc as a control. After 72 hours the cells were extracted and the levels of PP1γ expression monitored by western blotting. The results in Figure 4A show that loss of E6/E7 expression induces a marked increase in the total levels of PP1γ expression in HPV-16 positive cells, in a manner similar to that seen for restoration of p53 levels, which served as a positive control for efficient ablation of E6/E7 expression. We also monitored the efficiency of E6/E7 knockdown by RT-PCR and found that

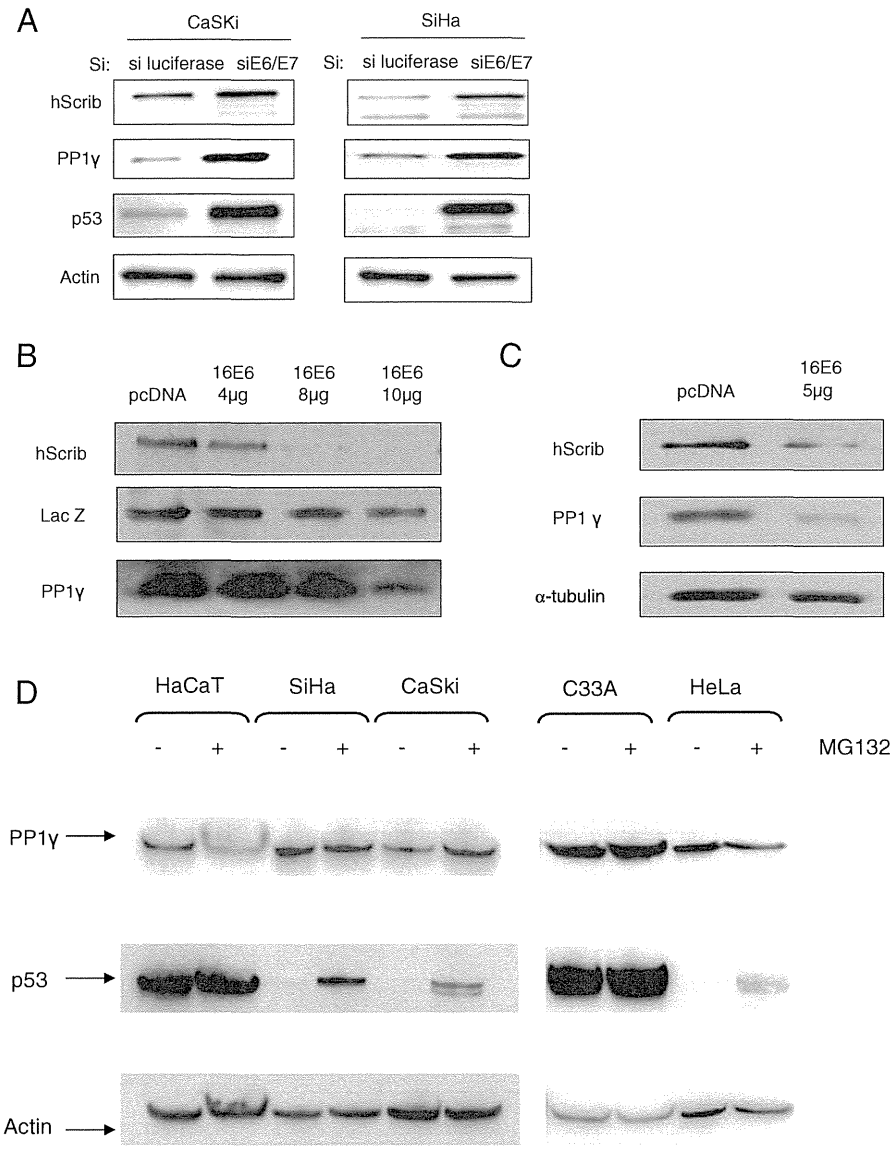

Figure 4 PP1γ levels are downregulated in HPV16 positive cells by HPV E6/E7 oncogenes. (A) HPV-16 positive CaSki and SiHa cells were transfected with siRNAE6/E7 or siLuc as control. Total cell extracts were then made after 72 hours, and hScrib, PP1γ, p53 and Actin were detected by western blotting. **(B)** 293 cells were transfected with 4, 8, 10 μg of HPV-16 E6 expression plasmid, and hScrib and PP1γ were analysed by Western blotting. The middle panel shows the LacZ transfection efficiency and loading control. **(C)** HaCaT cells were transfected with 5 μg of HPV-16 E6 expression plasmid, and hScrib and PP1γ were analysed by Western blotting. Tubulin was detected as control. **(D)** CaSki, SiHa, HeLa, C33A and HaCaT cells were incubated in the presence of either 10 μM MG132 or solvent before harvesting and analysed by western blotting. Actin was used as a loading control.

E6/E7 transcripts were reduced by around 60% following siRNA transfection (data not shown). In contrast to the change in PP1γ protein levels, we found no difference in PP1γ transcript levels after siRNA E6/E7 treatment in HPV-16 positive cells. Furthermore, to determine whether the cell type or E6 expression contributed to the alterations in PP1γ expression levels, we compared the ability of E6 to direct the degradation of PP1γ in 293 and HaCaT cells. First 293 cells were transfected with increasing amounts of HPV-16 E6, as indicated in Figure 4B. Then, we performed the same analysis using HaCaT cells (Figure 4C). The results demonstrated that overexpression of HPV-16 E6 results in a decrease in the level of PP1γ expression. In order to determine whether the loss of PP1γ expression was proteasome-mediated HPV-positive SiHa, CaSki and HeLa cells, and HPV-negative C33A and HaCaT cells were grown in the presence of the proteasome inhibitor, MG132 for 3 hours, after which the

cells were harvested and the levels of PP1γ expression ascertained by western blotting. As can be seen from Figure 4D, there are minimal changes in the levels of PP1γ expression following proteasome inhibition, regardless of the presence or absence of HPV DNA sequences, whilst there is efficient rescue of p53 following proteasome inhibition in HPV positive cells. These results indicate that the effects of E6 upon PP1γ patterns of expression are most likely proteasome independent.

Discussion

PP1 is a major serine/threonine protein phosphatase, normally regulating the phosphorylation status of a large number of important cellular regulatory proteins [36-39]. Important activities include the regulation of chromosome structure during mitosis and also following DNA damage, through de-phosphorylation of histones [30,40], and the control of centrosome disjunction through antagonism of Nek2A kinase activity [41].

In this study we have identified PP1γ as a potential new biomarker of HPV-16 induced malignancy. Using HPV-16 positive cervical tumour derived cell lines, IHC analysis of HPV-16 positive cervical tumours and CIN lesions, we present compelling evidence that PP1γ expression patterns are perturbed as a result of infection with HPV-16.

We originally considered that the PP1γ/hScrib complex might be a general target for HPV-16 E6, based on our previous studies showing complex formation between hScrib and PP1γ. However, analysis of the expression patterns of PP1γ and hScrib in cervical tissues indicate that this is not the case. Most importantly however, this highlights PP1γ as an independent target of the HPV-16 oncoproteins. In the normal cervix, PP1γ is expressed throughout the differentiating cervical epithelium, with a predominantly nuclear pattern of expression, which is consistent with previous studies [42]. To our surprise, we found that in all the HPV-16 positive cervical tumours analysed, this nuclear localisation of PP1γ was undetectable. Low levels of PP1γ can still be found within the cytoplasm of many cells within the majority of the cervical tumours that we analysed, although in 2/11 cases all PP1γ expression appeared to be lost. Similarly, perturbation in the pattern of PP1γ expression is apparent in CIN2 lesions, and this becomes more marked as the lesions progress to CIN3, suggesting that perturbation in the pattern of PP1γ expression is an early event in the development of cervical malignancy.

In order to understand whether these effects on PP1γ expression patterns were a direct consequence of E6/E7 activity, we then focused our attention on cells derived from HPV-16 positive cervical tumours. Again we found striking parallels with the IHC data, with very little PP1γ expression in the nucleus of HPV-16 positive CaSki or SiHa cells. In contrast, readily detectable nuclear PP1γ was observed in HaCaT cells. Most strikingly, siRNA ablation of E6/E7 expression resulted in a dramatic rescue of PP1γ expression within the nucleus of the HPV-16 positive cells, which appeared very similar to the effects seen upon the pattern of p53 expression. In contrast to p53 however, the alteration in the levels and pattern of PP1γ expression by E6 does not appear to involve the proteasome in cells derived from cervical tumours. Obviously further studies will be required to elucidate the precise mechanisms by which HPV-16 targets PP1γ.

Conclusions

Currently we have no information as to whether the HPV-16 E6/E7 oncoproteins can modulate any of these phosphorylation events in a PP1γ dependent manner, it is nonetheless intriguing that all of these pathways are perturbed to some extent in cells containing the HPV-16 oncoproteins. Future studies will investigate these aspects further, but it is tempting to speculate that targeting of the nuclear forms of PP1γ might contribute directly towards the generation of genome instability, chromatin remodeling and tumour progression. The cellular redistribution of PP1γ seems to have an important role in the development of centrosome abnormalities and chromosomal instability at early stages of cervical carcinogenesis. Taken together this study highlights the potential value of PP1γ as a novel biomarker for HPV-induced cervical neoplasia.

Competing interests

All the authors declare no competing interests.

Authors' contributions

TS performed the experiments and wrote the manuscript. KN (corresponding author) and LB supervised the experiments and wrote the manuscript. TS, KN, CK, KK, DM, HK-N, AT, YM, TA, OH-W, KO, SN, TY, MF, LB, YO, and TF contributed reagents, materials, experimental techniques, and data analysis. KN, DM, MF contributed pathological evaluation. All authors read and approved the final manuscript.

Acknowledgement

The authors are grateful to Kei Sakuma (Department of Pathology, Graduate School of Medicine, The University of Tokyo) for technical support on the preparation of IHC staining. Additionally, we thank Michihiro Tanikawa, Yuichiro Miyamoto, Kenbun Sone, Yuriko Uehara, Yuji Ikeda, Aki Miyasaka, Takahiro Koso, Tomoko Kashiyama, Tomohiko Fukuda, Kanako Inaba, Satoko Kojima, and Kensuke Tomio for their support and assistance. We also gratefully acknowledge the particular assistance of all members in Lawrence Banks's lab, and valuable comments on the manuscript from Miranda Thomas and David Pim. This work was supported by a Grant-in-Aid for Scientific Research (K.N.) from the Ministry of Education, Science and Culture, Japan, and in part by a research grant from the Associazione Italiana per la Ricerca sul Cancro (L.B).

Author details

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, The University of Tokyo, Tokyo 113-8655, Japan. [2]International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano-99, I-34012 Trieste, Italy. [3]Department of Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan. [4]Department of Obstetrics and Gynecology, Graduate School of Medicine, Teikyo University, Tokyo 173-8605, Japan. [5]Department of Obstetrics and Gynecology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan.

Received: 31 May 2014 Accepted: 27 February 2015
Published online: 07 April 2015

References

1. Zur HH. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2:342–50.
2. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. WHO international agency for research on cancer monograph working group. A review of human carcinogens–part B: biological agents. Lancet Oncol. 2009;10:321–2.
3. Butz K, Ristriani T, Hengstermann A, Denk C, Scheffner M, Hoppe-Seyler F. siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. Oncogene. 2003;22:5938–45.
4. Yoshinouchi M. *In vitro* and *in vivo* growth suppression of human papillomavirus 16-positive cervical cancer cells by e6 siRNA. Mol Ther. 2003;8:762–8.
5. McLaughlin-Drubin ME, Meyers J, Munger K. Cancer associated human papillomaviruses. Curr Opin Virol. 2012;2:459–66.
6. Mantovani F, Banks L. The human papillomavirus E6 protein and its contribution to malignant progression. Oncogene. 2001;20:7874–87.
7. Huh K, Zhou X, Hayakawa H, Cho J-Y, Libermann TA, Jin J, et al. Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. J Virol. 2007;81:9737–47.
8. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell. 1993;75:495–505.
9. Schlegel R, Phelps WC, Zhang YL, Barbosa M. Quantitative keratinocyte assay detects two biological activities of human papillomavirus DNA and identifies viral types associated with cervical carcinoma. EMBO J. 1988;7:3181–7.
10. Barbosa MS, Schlegel R. The E6 and E7 genes of HPV-18 are sufficient for inducing two-stage *in vitro* transformation of human keratinocytes. Oncogene 1989:1529-32.
11. Munger K, Phelps WC, Bubb V, Howley PM. The E6 and E7 genes of the human papillomavirus type 16 together Are necessary and sufficient for transformation of primary human keratinocytes. J Virol. 1989;63:4417–21.
12. Song S, Liem A, Miller JA, Lambert PF. Human papillomavirus types 16 E6 and E7 contribute differently to carcinogenesis. Virology. 2000;267:141–50.
13. Shai A, Nguyen ML, Wagstaff J, Jiang Y-H, Lambert PF. HPV16 E6 confers p53-dependent and p53-independent phenotypes in the epidermis of mice deficient for E6AP. Oncogene. 2007;26:3321–8.
14. Thomas M, Narayan N, Pim D, Tomaić V, Massimi P, Nagasaka K, et al. Human papillomaviruses, cervical cancer and cell polarity. Oncogene. 2008;27:7018–30.
15. Pim D, Banks L. Interaction of viral oncoproteins with cellular target molecules: infection with high-risk vs low-risk human papillomaviruses. APMIS. 2010;118:471–93.
16. Kiyono T, Kiyono T, Hiraiwa A, Fujita M, Hayashi Y, Akiyama T, et al. Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the Drosophila discs large tumor suppressor protein. Proc Natl Acad Sci U S A. 1997;94:11612–6.
17. Lee SS, Weiss RS, Javier RT. Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein. Proc Natl Acad Sci U S A. 1997;94:6670–5.
18. Gardiol D, Kühne C, Glaunsinger B, Lee SS, Javier R, Banks L. Oncogenic human papillomavirus E6 proteins target the discs large tumour suppressor for proteasome-mediated degradation. Oncogene. 1999;18:5487–96.
19. Nakagawa S, Huibregtse JM. Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. Mol Cell Biol. 2000;20:8244–53.
20. Glaunsinger BA, Lee SS, Thomas M, Banks L, Javier R. Interactions of the PDZ-protein MAGI-1 with adenovirus E4-ORF1 and high-risk papillomavirus E6 oncoproteins. Oncogene. 2000;19:5270–80.
21. Kranjec C, Banks L. A systematic analysis of human papillomavirus (HPV) E6 PDZ substrates identifies MAGI-1 as a major target of HPV type 16 (HPV-16) and HPV-18 whose loss accompanies disruption of tight junctions. J Virol. 2011;85:1757–64.
22. Bilder D, Li M, Perrimon N. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science. 2000;289:113–6.
23. Zhan L. Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. Cell. 2008;135:865–78.
24. Dow LE. Loss of human Scribble cooperates with H-Ras to promote cell invasion through deregulation of MAPK signalling. Oncogene. 2008;27:5988–6001.
25. Nagasaka K, Pim D, Massimi P, Thomas M, Tomaić V, Subbaiah VK, et al. The cell polarity regulator hScrib controls ERK activation through a KIM site-dependent interaction. Oncogene. 2010;29:5311–21.
26. Pearson HB, Perez-mancera PA, Dow LE, Ryan A, Tennstedt P, Bogani D, et al. SCRIB expression is deregulated in human prostate cancer, and its deficiency in mice promotes prostate neoplasia. J Clin Invest. 2011;121:4257–67.
27. Elsum I a, Yates LL, Pearson HB, Phesse TJ, Long F, O'Donoghue R, Ernst M, Cullinane C, Humbert PO. Scrib heterozygosity predisposes to lung cancer and cooperates with KRas hyperactivation to accelerate lung cancer progression *in vivo*. Oncogene 2013;:1–11.
28. Nakagawa S, Yano T, Nakagawa K, Takizawa S, Suzuki Y, Yasugi T, et al. Analysis of the expression and localisation of a LAP protein, human scribble, in the normal and neoplastic epithelium of uterine cervix. Br J Cancer. 2004;90:194–9.
29. Nagasaka K, Seiki T, Yamashita A, Massimi P, Subbaiah VK, Thomas M, et al. A novel interaction between hScrib and PP1γ downregulates ERK signaling and suppresses oncogene-induced cell transformation. PLoS One. 2013;8:e53752.
30. Shimada M, Haruta M, Niida H, Sawamoto K, Nakanishi M. Protein phosphatase 1γ is responsible for dephosphorylation of histone H3 at Thr 11 after DNA damage. EMBO Rep. 2010;11:883–9.
31. Trinkle-mulcahy L, Andrews PD, Wickramasinghe S, Sleeman J, Prescott A, Lam YW, et al. Time-lapse imaging reveals dynamic relocalization of PP1γ throughout the mammalian cell cycle. Mol Biol Cell. 2003;14:107–17.
32. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol. 1988;106:761–71.
33. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Hildesheim A, Schiffman MH, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol. 2000;38:357–61.
34. Thomas M, Massimi P, Navarro C, Borg J, Banks L. The hScrib/Dlg apico-basal control complex is differentially targeted by HPV-16 and HPV-18 E6 proteins. Oncogene. 2005;24:6222–30.
35. Gardiol D, Zacchi A, Petrera F, Stanta G, Banks L. Human discs large and scrib are localized at the same regions in colon mucosa and changes in their expression patterns are correlated with loss of tissue architecture during malignant progression. Int J Cancer. 2006;119:1285–90.
36. Bollen M. Combinatorial control of protein phosphatase-1. Trends Biochem Sci. 2001;26:426–31.
37. Cohen PTW. Protein phosphatase 1 – targeted in many directions. J Cell Sci. 2002;115:241–56.
38. Margolis SS, Walsh S, Weiser DC, Yoshida M, Shenolikar S, Kornbluth S. PP1 control of M phase entry exerted through 14-3-3-regulated Cdc25 dephosphorylation. EMBO J. 2003;22:5734–45.
39. Wu JQ, Guo JY, Tang W, Yang CS, Freel CD, Chen C, et al. PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. Nat Cell Biol. 2009;11:644–51.
40. Vagnarelli P, Ribeiro S, Sennels L, Sanchez-Pulido L, de Lima AF, Verheyen T, et al. Repo-Man coordinates chromosomal reorganization with nuclear envelope reassembly during mitotic exit. Dev Cell. 2011;21:328–42.
41. Mardin BR, Agircan FG, Lange C, Schiebel E. Plk1 controls the Nek2A-PP1γ antagonism in centrosome disjunction. Curr Biol. 2011;21:1145–51.
42. Andreassen PR, Lacroix FB, Villa-Moruzzi E, Margolis RL. Differential subcellular localization of protein phosphatase-1 alpha, gamma1, and delta isoforms during both interphase and mitosis in mammalian cells. J Cell Biol. 1998;141:1207–15.

3. 主な治療法と日常生活管理の実際

治療のキーポイント
1. 肺炎球菌のペニシリン耐性・マクロライド系薬耐性が増加している
2. ペニシリン耐性肺炎球菌にはペニシリン系薬の高用量投与が有効である
3. その他にセフェム系，ニューキノロン系，カルバペネム系薬なども有効である

肺炎球菌感染症ではペニシリン系薬が第一選択であるが，耐性菌が徐々に増加している．マクロライド系薬耐性が本邦では70%前後と高率である．

4. 病気を予防するための方策

幼小児や高齢者，脾臓摘出後などの患者には肺炎球菌ワクチンが有効である．

（笠原　敬，三笠桂一）

B グラム陰性球菌感染症
Gram-negative coccal infection

I 髄膜炎菌感染症 meningococcal infection

1. 髄膜炎菌感染症とはどういう病気か

髄膜炎菌 *Neisseria meningitidis* による感染症は，最も典型的には，髄膜炎菌性髄膜炎として発症する．

本症は，急性に発熱，激しい頭痛，悪心・嘔吐，項部硬直で発症する．劇症型の髄膜炎菌性菌血症は，発症数時間後に過半数の症例で死の転帰をとる．本菌は，鼻咽頭常在性のあるグラム陰性双球菌で，飛沫感染するが，通常は単なる不顕性感染か急性鼻咽頭炎として終わる．進展した病態としての髄膜炎菌性髄膜炎は感染症法5類の全数把握疾患である侵襲性髄膜炎菌感染症に含まれるが，わが国では散発にとどまる．小児の接触者には予防的抗菌薬投与を行う．

2. どのように考えて診断をつけていくか

診断のキーポイント
1. 高熱，皮膚，粘膜の出血斑，続いて頭痛，吐き気，精神症状，項部硬直
2. 頭痛，けいれん，意識障害からショックに陥り，電撃的に死に至る場合もあり

髄膜炎の診断には，発熱と上記の中枢神経症状を見極めることが肝要である．髄膜炎菌は，患者の血液・髄液から分離される．PCR法による菌の同定は，抗菌化学療法をすでに始めている場合や回復期にも用いることができる．髄液所見として圧上昇，多形核白血球増加，糖減少，蛋白質増加がみられる．

3. 主な治療法と日常生活管理の実際

化膿性髄膜炎を適応疾患とし，髄膜炎菌を適応菌種としているのはカルバペネム系のメロペネムであり，エンピリック療法には，本薬が第一選択となる．重症患者は，ICUで治療する．ショック例では救命的な処置が必要であり，頭蓋内圧亢進には，マンニトールを点滴する．播種性血管内凝固 disseminated intravascular coagulation（DIC）の診断・治療にも注意を払うべきである．

4. 病気を予防するための方策

髄膜炎菌ワクチンを接種することが，その予防対策であるが，わが国では発生率の低さからワクチンは認可されていない．アフリカなどの髄膜炎菌性髄膜炎多発地域への旅行者でワクチン接種を希望するものは，海外から個人輸入するか，海外で接種する以外にない．近年，国内大都市ではワクチン外来を開設して自費接種してくれる施設もある．

メモ 髄膜炎菌ワクチン：髄膜炎菌ワクチンとしては2005年にアメリカで承認された4価の結合型ワクチン（MCV4）が推奨される．これでは，病原性を持つ主な5群の髄膜炎菌のうち，A，C，Y，W-135の4群に対しては，長期持続予防効果がある．

Ⅱ 淋菌感染症 gonococcal infection

1. 淋菌感染症とはどういう病気か

淋菌 *Neisseria gonorrhoeae* による感染症で，男性の尿道炎と女性の子宮頸管炎が最も多い．

最近，オーラルセックスの増加に伴い，咽頭での保菌や感染が問題となっている．

淋菌感染症での最も大きな問題は，βラクタム，テトラサイクリン，キノロン耐性などが増加している点である．後述する3薬剤には現時点でほとんど耐性菌が認められていない．

2. どのように考えて診断をつけていくか

男性の尿道炎では，排尿痛，膿性尿道分泌物が出現，尿中白血球も認められる．女性の子宮頸管炎では症状を生じにくい．淋菌の検出法は，グラム染色鏡検，分離培養，核酸増幅法がある．男性は初尿，女性は子宮頸管分泌液を検体とする．

性器に淋菌が検出されたら，咽頭の検査も必要となる．検体は綿棒擦過または生食水うがい液とする．咽頭の淋菌検査には培養法が勧められる．New York City 培地やトリメトプリム含有 Thayer-Martin 培地を用いる．核酸増幅法のうち SDA 法と TMA 法は検出に有用である．

3. 主な治療法と日常生活管理の実際

1～3日程度の服薬で確実に除菌できる経口抗菌薬は皆無といってよい．わずかに下記の3種類の注射用抗菌薬が，著効を呈する．

・抗菌薬の選択と使用法

〈淋菌性尿道炎・子宮頸管炎〉

セフトリアキソン：静注　1.0 g 単回

セフォジジム：静注　1.0 g 単回

スペクチノマイシン：筋注　2.0 g 単回

〈淋菌性咽頭感染症〉

セフトリアキソン：静注　1.0 g 単回

4. 病気を予防するための方策

妊娠を望まない性行為においては，必ずコンドームを最初から着用することが肝要である．オーラルセックスにおいても，コンドームをつけるか，ラップフィルムのようなもので，バリアを設定すべきである．

メモ 淋菌性尿道炎および子宮頸管炎：男性の場合，淋菌性尿道炎を放置すると，淋菌性精巣上体炎に進展することがあり，本症が両側性に起こると，閉塞性男性不妊につながる．女性の子宮頸管炎も卵管炎，さらには骨盤腹膜炎に波及すると，発熱・疝痛をきたし，抗菌薬により軽快しても不妊症に陥ることがある．

（荒川創一）

C グラム陰性桿菌感染症 Gram-negative bacillary infection

Ⅰ 大腸菌感染症 *Escherichia coli* infection

1. 大腸菌感染症とはどういう病気か

a. 原因と病態

大腸菌は腸管内に常在するグラム陰性桿菌であり，市中感染や医療関連感染の原因となる．腸管外に漏れ出た場合に起こる腸管外感染症と，毒素や接着因子をもつ菌により腸管に起こる腸管感染症の二つに分けて考える．

b. 腸管外感染症

大腸菌は腸管外において以下のような感染症を起こしうる．

1）尿路感染症

市中感染の尿路感染症の原因として最多であり，膀胱炎，急性腎盂腎炎，前立腺炎などの原因となる．

2）腹腔内感染症

胆嚢炎，胆管炎，肝膿瘍，特発性細菌性腹膜炎，消化管穿孔後の二次性腹膜炎などの腹腔内感染症の原因となる．

3）その他

新生児の髄膜炎，入院患者の院内肺炎，皮膚軟部組織感染症などの原因となる．

c. 腸管感染症

下痢原性大腸菌は下記の5種類に分類される．

② 第1期・第2期早期潜伏梅毒

Px 処方例

ペニシリンGベンザチン注　1回5万単位/kg（最大240万単位）　1日1回　筋注（国内未承認：適宜ビクシリン注　1日100～150 mg/kg　静注などを使用する）

③ 晩期潜伏梅毒または期間不明の潜伏梅毒

Px 処方例

ペニシリンGベンザチン注　1回5万単位/kg（最大240万単位）　筋注　1週間ごとに3回（総量で15万単位/kg，最大720万単位）（国内未承認：適宜ビクシリン注　1日100～150 mg/kg　静注などを使用する）

④ 神経梅毒

Px 処方例

ペニシリンGカリウム注　1回5～7.5万単位/kg（最大2,400万単位）　1日4～6回　点滴静注　10～14日間

淋菌感染症

Neisseria gonorrhoeae infection

感5類

三鴨廣繁　愛知医科大学大学院主任教授・臨床感染症学

病態

- グラム陰性双球菌である淋菌（*Neisseria gonorrhoeae*）は，発育に炭酸ガス要求性で，栄養要求性が高い．また，日光，乾燥や温度の変化，消毒薬で簡単に死滅する．
- 性的接触が主な感染経路であるが，新生児淋菌性結膜炎は産道感染の結果として発症する．小児淋菌感染症では，性的虐待の可能性を疑うことは重要である．
- 口腔性交の増加により咽頭感染は増加傾向にあるが，無症状であることが多く，感染源となりうる．[m] 淋菌感染症はクラミジア感染を合併する頻度も比較的高い．
- 淋菌感染症の診断は，分泌物，擦過検体，咽頭拭い液（スワブ），口腔内うがい液などのグラム染色・鏡検法，培養，核酸増幅検査法（PCR法，SDA法，TMA法など）による．
- 代表的な小児淋菌感染症として，新生児淋菌性結膜炎，淋菌性尿道炎（男児），淋菌性子宮頸管炎（女児），淋菌性膣炎（女児），淋菌性咽頭感染，淋菌性急性精巣上体炎（男児），淋菌性骨盤内炎症性疾患（女児），播種性淋菌感染症，淋菌性直腸炎があげられる．

A 新生児淋菌性結膜炎

- 新生児淋菌性結膜炎は，産道感染により出生2～5日後に化膿性滲出液を伴って新生児に発症するもので，角膜潰瘍をきたし失明率も高い．

B 女児

- 性成熟前の女児は，性成熟した女性と比較してエストロゲン産生量が少なく，粘液産生能も低いことから，膣内はアルカリ性に傾き，淋菌が増殖しやすい環境にある．性成熟前の淋菌性膣炎は，通常，症候性であり，陰部は発赤し，大量の膿性分泌物，排尿障害，陰部不快感，疼痛を認める．同時に，肛門直腸や咽頭扁桃に淋菌のコロナイゼーションを認める．淋菌感染症は，適切な治療を行えば軽快するが，治療が行われなければ，急性症状は数日から数週間持続する．
- 上行性感染による卵管炎など骨盤内炎症性疾患は非典型的であるが，腹膜炎に至る症例もみられる．

C 男児

- 性成熟前の男児でも，成人男性と同様に尿道炎を呈するが，通常，発熱は認めない．無症候性膿尿をみた場合には，淋菌やクラミジア感染症も考える．

治療方針

テトラサイクリン系抗菌薬，キノロン系抗菌薬，ペニシリン系抗菌薬，多くのセフェム系抗菌薬の淋菌耐性率がきわめて高く，現在では，セフトリアキソン（ロセフィン），セフォジジム，スペクチノマイシンの3注射薬のみが臨床的に使用可能である（スペクチ

ノマイシンは，淋菌性咽頭感染症と播種性淋菌感染症では使用しない)．アジスロマイシンは，保険適用があるが臨床的検討がないこと，耐性菌の報告が増加していることもあり推奨しない．

咽頭感染例では除菌が困難である場合もあり，薬剤投与後に咽頭淋菌消失の確認が必要である．

パートナー(性的虐待実施者を含む)の診断，治療は必須である．

新生児淋菌性結膜炎の予防として，1%硝酸銀，エリスロマイシンやテトラサイクリンの眼軟膏や点眼薬が用いられる．眼感染症例では，局所製剤単独治療が無効であることが多く，必ず全身的治療薬である注射薬を用いる．

Rx 処方例

ロセフィン注　1回25～60 mg/kg(最大120 mg/kg　1日2回に分けて)　単回投与(精巣上体炎，骨盤内炎症性疾患，咽頭感染，播種性感染では1日2回や3～7日間投与する場合もある)　静注または点滴静注，もしくは筋注

レプトスピラ症

leptospirosis

感4類

吉田レイミント　長崎大学熱帯医学研究所准教授・小児感染症学

病態

- レプトスピラ症は，病原性レプトスピラ(*Leptospira interrogans*)の感染による人獣共通感染症である．病原性レプトスピラは保菌動物(主にネズミ類，イヌ，ブタ，ウシ)の腎臓に保菌され，尿中に排出される．ヒトには保菌動物の尿で汚染された水や土壌から経皮的または経口的に感染する．中南米，東南アジアなどの熱帯，亜熱帯地域に多い．日本では海外渡航者の増加に伴い，流行地からの輸入感染例を認める．
- 潜伏期間は約2～4日，多くは無症候性で90%は軽症型である．軽症型は発熱，悪寒，頭痛，倦怠感，眼球結膜充血，筋肉痛，腰痛などのインフルエンザ様症状を3～7日間認め，その後回復する．
- 約10%が重症型〔Weil(ワイル)症候群〕となり，発症5～8日後より黄疸，出血，肝・腎障害を認める．播種性血管内凝固症候群(DIC)，不整脈を伴う心筋炎を特徴とする多臓器不全，髄膜炎・髄膜脳炎，呼吸不全を伴う肺出血をきたす例もある．
- 保菌動物との接触や動物の尿に汚染された土壌や水への接触歴，流行地域への渡航歴を聴取する．抗菌薬投与以前発熱期の血液，尿，髄液を用いた，①培養(Korthof培地，EMJH培地など)，②レプトスピラDNA，*flaB*遺伝子検出(PCR法)，③急性期と回復期のペア血清で4倍以上の抗体価上昇を確認，のいずれかで確定診断する．

治療方針

レプトスピラ症を疑えばすみやかに抗菌薬治療を開始する．

A 軽症例

Rx 処方例　下記のいずれかを用いる．ただし，❶は9歳以上に，❷は9歳以下に用いる．

❶アクロマイシン末　1回7.5 mg/kg(成分量として)　1日4回(最大1日1 g)　7日間

❷サワシリン細粒(10%)　1回5～10 mg/kg(成分量として)　1日3～4回(最大1日90 mg/kg，成人量1回250 mg　1日3～4回)　7日間

❸ビクシリンドライシロップ(10%)　1回6.25～12.5 mg/kg(成分量として)　1日4回(成人量1回250～500 mg　1日4～6回)　7日間

B 重症例

Rx 処方例　下記のいずれかを用いる．

❹ペニシリンGカリウム注　1回5万単位/kg　1日4回　静注あるいは点滴静注　7～14日間(成人量1回30～60万単位　1日2～4回　筋注．なお年齢，症状によって適宜増減す

厚生労働科学研究費補助金
新興・再興感染症及び予防接種政策推進研究事業

性感染症に関する特定感染症予防指針に基づく対策の推進に関する研究

（H27－新興行政－一般－001）

平成27年度　総括・分担研究報告書

2016年3月31日発行

研究代表者　荒　川　創　一

連絡先　神戸大学医学部附属病院　感染制御部
〒650-0017　神戸市中央区楠町7-5-2
TEL. 078-382-5531（直通）　FAX. 078-382-6611